PLATE I.

APPARATUS FOR MAKING PHOTO-MICROGRAPHS.

1 2 3 4

1. Heliostat, to be placed on shelf outside of window.
2. Tube, to be placed in darkened window, having eight-inch lens at outer extremity, and ammonia-sulphate of copper cell at inner extremity.
3. Microscope.
4. Frame, containing ground-glass screen upon which the image is projected for photographing.

Heliotype Printing Co., Boston.

PHOTO-MICROGRAPHS

AND HOW TO MAKE THEM.

Illustrated by Forty-seven Photographs of Microscopic Objects,

PHOTO-MICROGRAPHS,

Reproduced by the Heliotype Process.

BY

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PREFACE.

In this little volume the Author has attempted to give such an account of the technique of Photo-micrography as will enable persons familiar with the use of the microscope to make photo-micrographs of suitable objects with a minimum expenditure of time and money. The Illustrations have been selected with a view to showing the kinds of microscopic objects best suited for photographing, and the results which may be expected by one who is willing to devote a little time to the mastering of technical difficulties.

The work is intended for beginners, and not for those who, having made some progress, are ambitious to undertake the more difficult feats in photo-micrography, — such as the photographing of difficult test-diatoms by oblique light. The Author has himself found no time for work of this kind, but has resorted to photography chiefly for the purpose of making pictorial memoranda of his microscopical observations. His scrap-albums are

filled with these photographic memoranda, and he constantly finds these useful for reference. Some also, reproduced by the heliotype process, have served to illustrate his papers relating to bacterial organisms. It is in the belief that others may find it worth while to avail themselves of this method of illustration that he has been induced to record the results of his own experience; and the aim has been, as far as possible, to simplify the technique and popularize the art.

In describing the plates, the Author has allowed himself considerable latitude, in the belief that a popular account of the objects described may lend interest to the book for some of those into whose hands it may fall, by reason of their unfamiliarity with the revelations of the microscope, and that such persons may perhaps have their interest aroused in microscopical studies, and be stimulated to further researches.

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PHOTO-MICROGRAPHS.

INTRODUCTION.

PHOTO–MICROGRAPHS are sun-pictures of microscopic objects more or less magnified.

A MICRO–PHOTOGRAPH is a microscopic picture of an object which can be seen by the naked eye.

These two names should not be confounded, as they are required to distinguish two very different things. By means of photo-micrographs, those unfamiliar with the use of the microscope may be made acquainted with a variety of beautiful and interesting objects which are completely beyond the reach of ordinary vision; while micro-photographs are simply curiosities in the art of photography, and are to be viewed under the microscope.

The art of making photo-micrographs is of recent origin, and in no part of the world has more been done to perfect the technique of this art, than in our own country. The foremost place among those who have aided in this progress will readily be conceded to Lieutenant-Colonel J. J. Woodward, a distinguished surgeon in the United States Army, whose photographs of difficult test-diatoms and of

artificially ruled lines will long serve as models which less accomplished microscopists can scarcely hope to equal; for success in making photo-micrographs depends upon the skill of the operator as a microscopist, and not simply as a photographer.

Indeed, the photographic part of the work may be delegated to another, and still the microscopist is properly credited with the result, as the merits of the picture, *as a photo-micrograph*, depend upon his skill, in preparing the object to be photographed and projecting its image upon the screen; while its merits as a *photograph* depend upon the technical skill of the operator who makes a permanent record of this image. This is done by the ordinary processes of photography, and it will be very desirable that the amateur who proposes to make photo-micrographs and, as must commonly be the case, to be his own photographer, should have a few lessons from a practical photographer. It is true that this part of the work is greatly simplified by the use of dry plates; but even with these, and with explicit printed directions with reference to their use, the beginner is pretty sure to expend a considerable amount of time and material, if he trusts to his unaided efforts, in experiments relating to time of exposure, development, etc.

In the present volume, attention will be chiefly directed to that part of the art of making photo-micrographs which is the work of the microscopist, and the reader is referred to the nearest practical

photographer, or to a manual of photography, for detailed instructions with reference to the processes of wet-plate photography, silver-printing, etc. Directions will be given, however, for the dry-plate process, which offers especial advantages for this purpose, and particularly for those who, being engaged in other pursuits, can only devote a day now and then to photo-micrography.

The advantages of dry plates are as follows: They can be purchased ready for use in such quantities and of such sizes as may be desired; they may be kept indefinitely without deterioration; no time is lost in preparing for work; they are more sensitive than wet plates; they can be developed at any time subsequent to exposure; and, finally, they do not stain the operator's fingers.

The plates which I have used most extensively, and with which I have been well pleased, are manufactured by the Eastman Dry-Plate Co., of Rochester, N. Y.

Sun-pictures, as compared with hand-work, have the advantage of being quickly made, and of being absolutely true to nature. The best-executed drawings or engravings are commonly more or less diagrammatic, and the highest skill is required to produce satisfactory illustrations of many of the most interesting and beautiful microscopic objects. But by photography, the most delicate lines and shades are preserved, and one who has no skill in drawing is able to make a permanent record of what he sees in the course of his microscopical researches. That is, *within certain limits*, for not every

microscopic object is suited for photography; and those who imagine that it will be a very simple matter to make photographs of all the objects in their collection when they have provided themselves with the necessary apparatus and have learned to develop a dry plate, are doomed to disappointment. Candor compels the admission that very many objects which seen under the microscope are very beautiful, cannot be successfully photographed. This is true of a majority of the histological and pathological preparations not mounted especially for this purpose. It is true as regards opaque objects, and objects of a deep red, yellow, orange, or brown color, as these are practically opaque for the actinic rays. In short, success in photo-micrography depends to a considerable extent upon the selection of suitable objects, and upon mounting these in the best manner for the purpose in view. Some hints and directions will be given in regard to this important matter in the portion of this volume devoted to technology.

The critics should remember in judging of the merits of a photo-micrograph, that it should not be compared with hand-work, which, intentionally or otherwise, is usually more or less diagrammatic or "conventionalized." On the other hand, it should be compared with the picture seen under the microscope. Those who are not familiar with the views from nature obtained in this way, and who are familiar with the woodcuts commonly employed for the illustration of works relating to

the microscope and its revelations, will be likely to prefer the neat appearance and the sharply drawn lines of the latter to a photograph from nature.

But for the trained eye, the photo-micrograph possesses special attractions; just as the photograph of a friend conveys impressions and gives rise to reminiscences of an agreeable nature, while that of a stranger is looked upon with indifference. The expert microscopist, also, knowing the difficulty of obtaining an ideal field free from extraneous objects, and having every portion in perfect focus, is willing to make some allowance for slight defects which at once attract the attention of a critic who is not a microscopist. In the same way, the connoisseur in art in looking at a famous old picture learns to overlook the stains and fissures imprinted by the hand of time, and to his mental vision the work of the master is revealed in all its beauty, while for the untrained eye the scarred and dingy canvas possesses no attractions.

By exercising great care and patience in the mounting of objects and in the selection of a field, all imperfections may be excluded except such as result from the physical characters of the object itself and the limits imposed by the laws of optics. In photographing with high powers, and especially with wide-angled objectives, it will be found that the slightest irregularity in the surface of an object makes it impossible to have all parts of the field in perfect focus at the same time. This is well shown in my photographs of *Triceratium favus*

(Plate XIII.). This diatom is slightly convex, and when the centre is in focus, the margins are not. This difficulty is best met by the use of objectives having the greatest possible penetrating power, — focal range, — and by focusing so as to obtain the best possible general result, viewing the field as a whole.

When a number of objects which differ in thickness, degree of transparency, or in color, are grouped together in the same field, we have to contend with a photographic difficulty arising from the fact that the time of exposure required to bring out the details is greater for certain of these objects than it is for others. Under these circumstances, the best we can do is to give such an exposure as will produce the best average result. Thus in my photograph of the fourth square of Möller's Typenplatte (Plate XII.), the thicker diatoms, such as *Eupodiscus Argus*, would be improved by a longer exposure, but this would destroy all the details in some of those which are thinner and more transparent.

Photo-micrographs are especially useful for the illustration of scientific papers and works requiring illustrations of this kind, of which only small editions are wanted.

By means of the heliotype process, prints may be obtained at short notice, which, being in printer's ink, are more durable than silver-prints, at a cost which is simply that of printing, for the gelatine plate from which the printing is done is inexpensive, and quickly made from any good negative,

which may be placed in the hands of the Heliotype Company.

Photo-micrographs also furnish a ready means of illustrating popular lectures, by the projection upon a screen of highly magnified microscopic objects.

These pictures from life have always a greater interest for those who are being introduced to the wonders of nature as revealed by the microscope, than have hand-made drawings.

Positives upon glass suitable for projection are made very quickly, and without a camera, by simply placing a dry plate at the back of the negative, exposing it to the light for an instant, and developing the image with the ferrous-oxalate solution.

PART I.

TECHNOLOGY.

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TECHNOLOGY.

I. THE LIGHT.

SUNLIGHT is the best and cheapest light for photography, and those who propose to make photo-micrographs are advised to make use of it in preference to any other, unless circumstances prevent them from working during the daytime, or they are in a locality where the full light of the sun can rarely be obtained, in consequence of clouds, fogs, or dense smoke.

Photographing Transparent Objects by Transmitted Light.

For powers up to one hundred diameters, light reflected from the clear blue sky and transmitted directly through the object, without the intervention of a reflecting mirror or condensing lens, is all that is required. Beginners are advised to make their first efforts with a simple apparatus arranged to use the light in this way. Not only is the necessary apparatus the cheapest and simplest possible, but the light is of the best quality. Being reflected from a perfect mirror, — the blue

sky, — the rays are practically parallel, and the complications are avoided which are introduced when a reflecting surface or refracting lens is interposed.

For higher powers, and after learning how to make good negatives with the simplest conditions possible, the student may place an achromatic condenser beneath the stage. An achromatic object-glass may be used for this purpose. The sub-stage of the microscope should be arranged to carry any objective having the Society's screw, and to enable the operator to adjust this at the proper distance from the object. This distance will depend upon the power of the condensing lens, and also upon that of the objective employed in front of the object to project the image.

When a condensing lens is used, it should be adjusted by trial to that point which gives the best definition to an object properly focused on the screen. If it be brought nearer to the object than this most favorable point, the illumination of the field upon the screen will be brighter, and the image may for this reason appear more distinct. But a careful inspection will show that there is loss of definition, and that the finer details of the projected image are obscured or entirely lost. This will be still more apparent in a negative made under these circumstances.

If the light is insufficient when the condenser is adjusted to the best point for definition, it will be necessary to place another condensing lens of larger diameter — a bull's-eye condenser may be

used — at a proper distance at the back of the achromatic condenser, or to use a heliostat.

In general terms, it may be said that any image upon the screen which makes a distinct impression on the retina in a dark room can be photographed.

If the light is very feeble, and the image barely perceptible, it will be necessary to use a very sensitive plate, and to make a long exposure.

The writer has made a good photograph of Bacteria (*B. rubescens*, Ray Lankester) with an amplification of six hundred diameters, without a heliostat, using a dry one-sixth-inch objective of Zeiss as a condenser, and obtaining the light directly from the blue sky. The objective used was Zeiss's one-eighteenth-inch homogeneous oil-immersion, and the time of exposure was half an hour, Eastman's instantaneous dry plate.

From five to six hundred diameters is probably about the limit with light reflected from the sky, and with such condensing apparatus as is commonly supplied by dealers in optical apparatus for ordinary use with the microscope. The writer can see no good reason why the opticians should not be able to construct an achromatic condenser for photography which by collecting the light from a larger area would give sufficient illumination for the highest powers, and thus enable the operator to dispense entirely with the use of a heliostat, which with its accessories is a somewhat troublesome and expensive piece of apparatus.

It must be remembered that the actinic power of the light from the sun varies greatly at differ-

ent times and in different places. It is less in northern latitudes and during the shortest days, when the sun's rays reach the earth in a very oblique direction. It fails rapidly as the sun sinks in the heavens towards evening. It is greatest when the atmosphere is transparent, and distant objects are distinctly seen. On the other hand, a hazy condition of the atmosphere indicates that a multitude of minute particles are suspended in it, and these being interposed between our mirror —the sky— and the object, obstruct and weaken the light.

The smoke of factories, etc., even when greatly attenuated, is a veil in front of our mirror which is fatal to any attempt to work except with the lowest powers, and even with these it introduces an element of uncertainty which leads to much trouble.

This smoke and haze is commonly confined to the lower regions of the atmosphere, and if our instrument is placed horizontally, so as to receive light reflected from the sky at a point near the horizon, we have the disadvantage of having a thick stratum of this hazy atmosphere interposed in front of our mirror. Moreover, unless we are in an elevated situation, a horizontal position of the instrument is apt to cause the condensing lens to face some terrestrial object, instead of the clear blue sky. It is therefore generally necessary to place the instrument obliquely with reference to the surface of the earth, in the position of a telescope, and the elevation must be greater or less, according to circumstances.

For convenience in working, it is desirable that the microscope should be as nearly horizontal as possible. To secure the best illumination, it is essential that it face the clear blue sky. Theoretically, those two conflicting requisites could be reconciled by the use of a plane mirror inclined at an angle of 45°, by which light from the sky overhead would be reflected in a horizontal direction; but in practice I have not found this to work so well, especially when no condensing lens is employed, for the reasons that there is a loss of light in changing its direction by a reflecting surface, and that imperfections in the mirror or dust upon its surface give rise to an unequally illuminated field. An apparatus mounted upon hinges in such a manner that it could be placed horizontally or more or less obliquely as required, would probably fulfil the conditions the most satisfactorily, as the adjustments could be made while it was in a horizontal direction, and the anterior extremity elevated, or the posterior extremity depressed, for the purpose of making the exposure. In this case, a final focusing would be necessary, after changing the position.

The writer has not found it necessary to resort to either of these expedients, but has been in the habit of working with his instrument permanently inclined at an angle of eight or ten degrees.

The instrument may be directed towards any point of the compass; but if it points south, the direct rays of the sun should be excluded by a short tube, blackened on the inside, arranged with

its axis in a line with that of the instrument, so that none but parallel rays reflected from the sky reach the object, or the back lens of the achromatic condenser, if one is employed.

It may be given as an absolute rule that *no light should reach the screen except that which comes from behind the object;* and it is always desirable to shut out, by means of diaphragms at the back of the object, all light not directly concerned in the illumination of the field to be photographed.

In photographing with comparatively high powers without a heliostat, wide-angled immersion lenses possess a special advantage, from the fact that they admit more light than lenses of less angular aperture having the same magnifying power.

It is evident that the light which enters the objective will be more or less attenuated according to the distance between the screen and the object-glass, and *this attenuation is as the square of the distance,* while the increase in magnifying power — in diameters — varies in a simple ratio, corresponding with the increase in distance. Thus if at a given distance the amplification is one hundred diameters, at twice this distance it will be two hundred, and the light will have but one fourth the intensity. This should be borne in mind in estimating the time of exposure required with the same lens and light, with the screen at a greater or less distance from the object-glass.

Under the law above stated, the attenuation of light becomes so great for high powers that illu-

mination from the clear blue sky is no longer sufficient, and it is necessary to use a heliostat.

This instrument carries a mirror, by which the direct rays of the sun are reflected in a line which corresponds with the axis of the achromatic condenser. A clock-work apparatus causes the mirror to follow the sun in such a manner that, when properly adjusted, the reflected light continues to fall in the same direction, notwithstanding the constantly changing position of the source of illumination. It is essential when working with a heliostat that the apparatus for making photo-micrographs be arranged in a window facing the south, for this instrument must be placed upon the meridian, with its mirror facing to the north. When adjusted for latitude, placed on the meridian in a perfectly level position, and set to the true time, the heliostat throws the light continuously upon one spot, if the clock is regulated with precision. But in practice, this is rarely accomplished, and a heliostat regulator is required to centre the light just before making an exposure; for if the light is not exactly centred, the field is unequally illuminated.

Not only the actinic rays of the sun, but the heat rays as well are reflected by the heliostat; and being brought to a focus by the achromatic condenser, they would quickly destroy the object and melt the balsam mountings of the lenses of the objective, if some way were not provided to arrest them. The problem is to arrest or throw aside the heat rays without interfering with the

actinic or chemical rays, which are at the violet end of the spectrum. This may be accomplished by means of a glass prism, which separates the rays by virtue of their different refrangibility; but in practice it is more conveniently accomplished by allowing the light to pass through a medium which is transparent for the actinic rays, but which arrests the heat rays.

The medium commonly employed is a solution of the ammonio-sulphate of copper. This is a liquid having a deep-blue color, and is easily made by dissolving sulphate of copper to saturation in strong water of ammonia. The solution is to be filtered, and is placed in a flat glass cell, having parallel sides, which is introduced between the mirror and the achromatic condenser. The sides of the glass cell should be separated by about half an inch, so that the light may pass through a stratum of the ammonio-sulphate solution of this thickness. The other dimensions of the cell are not of material importance, but two inches square will be found a convenient size.

Experience proves that it is well to introduce a lens of long focal distance between the mirror of the heliostat and the achromatic condenser. Dr. Woodward's regulator, which is recommended to those who desire to provide themselves with apparatus of this kind, is provided with an eight-inch lens carried at the outer extremity of a tube passing through the frame or plate of the regulator, while the inner extremity carries a metal pocket into which the ammonio-sulphate cell is introduced.

The writer would repeat the advice already given to those who propose to learn to make photo-micrographs, to commence with the simplest apparatus, and not to purchase a heliostat and accessories until a certain amount of practice has demonstrated the aptitude of the student for mastering technical difficulties, and his continued desire to photograph more difficult objects with higher powers.

Those who desire to purchase are recommended to buy the heliostat manufactured by Edward Kübel, of Washington, D. C. (No. 328 First Street, N. E.). This instrument, with the accessories, first made under the direction of Surgeon J. J. Woodward, United States Army, is especially adapted for the purpose, and it is doubtful whether a cheaper instrument, if one is made, would prove as satisfactory.

The price given by Mr. Kübel about a year ago is as follows: —

Keith's heliostat with extra size mirror . . .	$54.00
Woodward governor	36.00
Mounted actinic condensing lens	10.50
Blue cell	4.00
Blue cell holder and six diaphragms	7.00
Foot-plate for heliostat	1.75

In northern latitudes, and during the winter months, the sun is so low in the heavens that the mirror of the heliostat, being greatly inclined, gives only a narrow line of light. For this reason, and because of the diminished actinic power of the light, those who live in southern latitudes

have a decided advantage at this season of the year.

All the photo-micrographs illustrating this volume were made by *central light*, as the writer has given no personal attention to photography by oblique illumination, having been occupied chiefly in the practical work of making photographic memoranda of the microscopic objects met with in the course of his researches relating to the etiology of the infectious diseases.

For this purpose he has found central light all that was required, and he has had no ambition to follow Dr. Woodward and others in the difficult feats which they have accomplished in the way of photographing difficult test-diatoms, — e. g. *Amphipleura pellucida*, etc.

Those who have this ambition will find it necessary to resort to *oblique illumination*, and doubtless will do well to obtain Woodward's Oblique Illuminator, — an apparatus for obtaining oblique illumination at definite angles.[1]

Artificial light may be used for making photo-micrographs. In localities where the sun is obscured by clouds, fog, or dense smoke, during a considerable portion of the time, and for persons who are otherwise occupied during the daytime, and who desire to work at night, some kind of artificial light will be essential.

It is probable that the electric light will be found the most economical and convenient, if it is ever generally introduced for illuminating pur-

[1] Described in the Amer. Quart. Micr. Journal, Vol. I. pp. 268–272.

poses; but to employ an electric light for this object alone would involve an expense which few would care to incur. The same may be said of the magnesium and of the calcium lights. Dr. Woodward some years since demonstrated that all these lights may be used, but the result of his experiments was that sunlight is preferable, independently of the question of expense.

Photo-micrographs may also be made with a powerful oil-light, and for low powers this source of illumination should probably be placed next to sunlight for convenience and cheapness. The writer made some experiments a year and a half ago which satisfied him of the practicability of making good photo-micrographs in this way, and also of the superiority of sunlight.

Recently, Professor C. Henry Kain has obtained some very creditable results with an ordinary kerosene lamp and an amateur photographic outfit.[1] The editor of the journal in which Professor Kain recorded his results has also met with success in photographing by lamplight, and has written an interesting article upon the subject.[2]

Black-ground Illumination.

Black-ground illumination is effected by stopping out the light immediately behind the object by means of an opaque cement applied to the central portion of the front lens of the condenser.

[1] See the Amer. Month. Micr. Journal, Vol. III. No. 4, p. 71.
[2] Ibid., Vol. III. No. 5, pp. 88–92.

This "spot lens" being properly focused, the object is illuminated by a cone of oblique rays emerging from the uncovered margin of the front lens of the condenser. Carpenter says of this: "It is one of the great advantages of this kind of illumination that, as the light *radiates* from each part of the object as its proper source, instead of merely *passing through* it from a more remote source, its different parts are seen much more in their normal relations to one another, and it acquires far more the aspect of solidity."[1] This difference in the appearance of a transparent object, according as it is illuminated by ordinary transmitted light or by black-ground illumination, is illustrated in Plate XV. In Fig. 1 of this plate we have the diatom *Coscinodiscus oculis iridis* illuminated in the ordinary way, and in Fig. 1', the same diatom as seen by black-ground illumination. The true form of the object is brought out by the latter method, and the appearance of solidity spoken of by Carpenter is well shown; while in Fig. 1 we seem to be looking at a flat disk, of the thickness of which we are unable to judge.

Photographing by Reflected Light.

But little has been done in the way of making enlarged photographs of opaque objects by reflected light. This is probably due to the fact that, for any except the lowest powers, there are

[1] "The Microscope and its Revelations," sixth edition, p. 125.

technical difficulties which have not been solved in a satisfactory manner.

Photo-micrographs of the surfaces of opaque objects, of insects, foraminifera, etc., would be exceedingly interesting, and useful for illustrating scientific treatises, popular lectures, etc.

It is well known, in ordinary photography, that objects in different planes can only be brought into focus at the same time in a greatly reduced picture. In a life-size photograph of a man, for example, when the nose is in perfect focus, the ears will be out of focus, indistinct and distorted. This difficulty is overcome by making a reduced picture in the first instance, and then enlarging this to any extent desired. Minute objects present irregularities of form and of surface as great, relatively, as larger ones, and the difficulty referred to is magnified in proportion as we magnify the object.

The quality required of a lens which is best adapted to overcome this difficulty is called *penetrating power*. This quality increases with the focal distance of the objective. For this kind of work, therefore, it will be necessary to use low-power objectives, and those of comparatively small angular aperture. It will, no doubt, be found also that the best results are obtained by making a first-class negative with very moderate amplification, and enlarging from this to the dimensions desired.

In the case of flat surfaces, such as a butterfly's wing, the leaves of plants, etc., the difficulty

referred to is reduced to a minimum, and the writer has succeeded in making some very fair photographs from objects of this kind. Fig. 2, Plate IX., has been introduced as an illustration of what may be done in this way. It also illustrates the difficulty of securing uniform illumination, and of obtaining a field in which the definition is good throughout. We have the same difficulty with these minute stellate hairs on the leaf of *Deutzia scabra* that the portrait photographer has with the nose and ears of his subject. It is difficult to do justice to both.

The definition, also, is not as perfect as could be desired, although the objective used is one of the best in my collection (Tolles's two-inch). Another photograph by the same lens is given in Fig. 1, Plate VII. The general effect is very good, and we recognize the fact that these pollen grains are little spheres; but the minute spines which project in all directions from their surface can be seen only at the outer margin of the hemisphere facing us. These correspond with the ears of our man; and as the amplification is twenty-five diameters, the spines corresponding with the nose are as much out of focus as this organ would be in the face of a man magnified twenty-five times.

In my experiments with reflected light, illumination has been effected by means of a Lieberkühn, by which parallel rays reflected from the sky are concentrated upon the object. When the amplification is as much as twenty-five diameters, it is

evident that the light is greatly attenuated, and the time of exposure must be correspondingly long. If we suppose that the illumination would be sufficient to photograph the object in one second of time at its natural size, then the time required when it is magnified twenty-five diameters should be the square of twenty-five, or 625 seconds. As a matter of fact, the time required is considerably more than this, and some more efficient mode of illumination is required.

One plan which has suggested itself is to concentrate the light from a larger area upon the Lieberkühn by means of a metal or glass funnel silvered upon the inside.

Or artificial illumination might be employed by means of a platinum wire made incandescent by a powerful electric current, and arranged in a circle just behind the lower end of the objective in such a manner that the light would fall upon the object, and that only reflected light could enter the objective. A Lieberkühn behind the incandescent wire would be required as a reflector. It will be understood that these are merely suggestions which have not been put to the practical test.

II. MICROSCOPICAL APPARATUS.

All that is absolutely required in the way of microscopical apparatus in order to make a commencement, is a monocular stand of the simplest construction and a low-power objective.

For powers up to one hundred diameters, when sunlight reflected from the clear blue sky is employed, no condensing lens is required, and the beginner is advised to make his first efforts with the simple apparatus indicated, using a good one-inch objective, and selecting as the object to be photographed the cuticle of some plant, such as the lily or the house-leek, or a very thin transverse section of some succulent stem.

For powers above one hundred diameters, it will be advisable to use a condensing lens, and an ordinary one-fifth-inch objective will answer the purpose very well, when the object-glass used is anything below one-eighth-inch. It is best to have a little metal cap over the face of the condensing lens, with a small aperture in the centre which permits the passage of only so much of the light as is required for the illumination of the field, when the higher powers are used.

The sub-stage of the microscope should be provided with means for focusing the condenser forward and back.

A spring nose-piece is required when high powers are used.

A mechanical stage is not essential, even for work with the highest powers; but it is a great convenience, and one who proposes to do much work will scarcely be willing to dispense with it.

In the absence of a mechanical stage, it is important that the hand movement be very smooth and steady, as objects often require to be carefully centred for photography; and when a good field

has been found, it is very annoying to lose it in an attempt to move the slide a trifle one way or the other.

The stand should be very steady, the movement by the coarse adjustment smooth and regular, and the fine adjustment as delicate as possible.

It is of course essential that the body of the microscope be attached to the foot by a joint which permits of its being placed in a horizontal position. This joint must be firm, and there should be a substantial arrangement for stopping or holding the body in an exactly horizontal position.

An inner tube — draw-tube — is desirable, and this should be provided at its lower end with the Society's screw, to hold an amplifier, and also for the purpose of carrying a low-power objective, such as a four or six inch lens; for the working distance upon most stands is not sufficient to permit the use of an objective of this focal length at the end of the outer tube. This arrangement also permits the operator to attach a Lieberkühn to the extremity of the outer tube, and a low-power objective to that of the inner tube, for the purpose of photographing objects by reflected light.

The Lieberkühn and the objective may thus be focused separately, the former first, by the coarse adjustment, and the latter subsequently, by means of the draw-tube. The final focusing with the fine adjustment will not materially interfere with the position of the Lieberkühn, which should be that which illuminates the object most effectually.

The head of the fine-adjustment screw should be of as great a diameter as is practicable, and should be grooved around the margin for the purpose of carrying a cord, which will be required in focusing upon the screen.

In working with a heliostat and with the highest powers, a delicate centring apparatus for the achromatic condenser will be required, and all the movements of the stage and sub-stage with which first-class stands are provided will be desirable, if not absolutely necessary. The large stand of Powell & Lealand[1] is an excellent one for the purpose, and is that which is preferred by Woodward.

Objectives.

It is a delicate matter to recommend the objectives of certain makers, when there are so many competitors in the field; but in the interest of the purchasers of this little book, the Author proposes to give the results of his experience. No doubt a fair trial of the best work of makers not named would show that their objectives are equal to some of those for which a preference is expressed.

Beginners are advised at the outset to make their first efforts with such objectives as they may possess, and to remember that the most expensive objectives are not necessarily the best for their purpose. Some of the wide-angled modern objectives are practically useless for photography,

[1] See "The Microscope and its Revelations," sixth edition, p. 90.

on account of their short working distance. It must be remembered that a lens which has barely enough working distance to enable the operator to focus through the thin glass cover of the preparation he desires to photograph, when the eye-piece is used, will fail entirely when this is removed, and an attempt is made to project the image upon a screen removed to a distance of several feet. For *the farther the screen is removed from the object, the nearer the objective must approach it.* Objectives of moderate angle have the advantage of greater penetration and ample working distance. These are therefore to be preferred for ordinary use, and it is only when *resolving power* of a high order is required for special work, that the more expensive wide-angled lenses will be essential.

Flatness of field and good defining power are qualities which every first-class objective should possess; and when the Author a few lines back advised beginners to use such microscopical apparatus as they might possess, he referred to objectives of this kind, and not to the cheap toys which abound in the market, for it would be a waste of time to attempt to do anything with them. First-class objectives made ten or fifteen years ago, very suitable for photography, may often be obtained second hand at a very moderate price, as the tendency of late years has been to seek wide-angled lenses.

The following objectives by well-known makers are recommended, — the four-inch, two-inch, one-

inch, and one-half-inch (with cover correction) of Powell & Lealand, of London; the two-inch and four-tenths-inch of Tolles (Boston); the one-inch and one-fourth-inch of Collins (London); the two-thirds-inch and one-fifth-inch of Beck (London); the one-sixth-inch of Spencer (Geneva, N. Y.); the one-sixth-inch (DD), dry, and the one-eighth, one-twelfth, and one-eighteenth inch homogeneous immersion objectives of Zeiss (Jena, Germany). This list could no doubt be greatly extended, but the writer has refrained from recommending any objectives except such as he has himself practically tested in photography.

The homogeneous immersion objectives possess decided advantages, and the one-eighteenth of Zeiss is to be especially commended to those who require so high a power. It is doubtful whether, in the present state of optical science and art, it is worth while to look for anything superior to this. That other makers may equal it, is quite probable, and immersion objectives which have the high guaranty of coming from such makers as Powell & Lealand, of London, or Tolles, of Boston, may be purchased without hesitation. I have recently seen an immersion one-eighth by Gundlach, of Rochester, N. Y., with which I was much pleased. The great objection to the best American objectives is their cost. The water-immersion objectives of Hartnack are excellent, and are more conveniently used, especially for extemporaneous preparations, than are the homogeneous immersion lenses.

The oil of cedar, which was first introduced for use with the latter, has generally been abandoned, because of its solvent action on cements, and a viscid fluid has taken its place, consisting of some salt dissolved in glycerine, to obtain the proper refractive index.[1] This viscid fluid causes the thin glass cover to adhere so strongly to the front lens of the objective, that the cover itself, if not fastened by cement, or even the whole slide, is lifted when the attempt is made to focus upon the object. For this reason, dry or water-immersion lenses are more convenient for every-day use.

When working with a heliostat, an amplifier may be used in the tube, to increase the magnifying power. With a first-class amplifier, such as that manufactured for this purpose by Tolles, of Boston, the amplification is nearly doubled, without any marked loss of definition. The amplifier must be adjusted by means of the draw-tube with reference to the distance of the screen and the power of the objective.

[1] Hydrate of chloral dissolved in glycerine is perhaps the favorite immersion fluid.

III. CAMERA AND PHOTOGRAPHIC MATERIAL.

One of the small cameras furnished with the cheap photographic outfits for amateurs now in the market, may be used by beginners, or by those who propose to devote only an occasional spare hour to this kind of work. But it will be better for one who really intends to succeed, and to make a practical use of photography in connection with his microscopic studies, to purchase a well-made camera, the plate-holder of which will take plates of the following standard sizes, — $6\frac{1}{2} \times 8\frac{1}{2}$ inches; $4\frac{1}{4} \times 5\frac{1}{2}$; $3\frac{1}{4} \times 4\frac{1}{4}$; $2\frac{3}{4} \times 3\frac{1}{4}$.

The beginner is advised to use only the smallest plates, or the quarter-size ($3\frac{1}{4} \times 4\frac{1}{4}$), until he is sufficiently expert to be sure that he will get a first-class negative, at least occasionally. Unless he has the advantage of receiving a few lessons from a practical photographer, or has better fortune than the writer anticipates, there will be a considerable expenditure of dry plates before photo-micrographs will be produced which he is ready to pronounce quite satisfactory. The above hint is therefore given on the score of economy.

It will be safe to order a gross of quarter-size plates to start with, together with the necessary chemicals for developing and fixing. Fortunately, these are inexpensive, and not difficult to manipulate.

The following chemicals will be required, — protosulphate of iron, oxalate of potash, and hyposulphite of soda.

From one to five pounds of each may be purchased as a stock to start with. Two shallow trays for the developing and fixing solutions will also be required, and these may be of porcelain or of hard rubber; for the quarter-size plates, oblong glass dishes may be used, such as can be bought at any crockery store for a trifle. Nothing is said here of the photographic materials required for intensifying, developing by other methods, and making silver-prints.

The ferrous-oxalate developer gives as good results in photo-micrography as does any other with which the writer is acquainted, and it is desirable that the beginner should become thoroughly familiar with its use before experimenting with other developers.

Intensification is not needed when the negative is properly timed; and if not well timed it is better to reject it and make another.

Silver-prints can be obtained from the nearest photographer more economically than the amateur can make them himself. It is an advantage, also, to the beginner to take his negatives to a practical photographer to be printed, as he will receive valuable hints as to what constitutes a good printing negative, and will doubtless find that negatives with which he was very well satisfied at first, hardly come up to the standard.

IV. ARRANGEMENT OF MICROSCOPE AND CAMERA FOR PHOTOGRAPHY.

THE simplest arrangement, and that which is recommended when the operator is not able to fit up an operating-room for the special purpose of making photo-micrographs, is to attach the microscope, placed horizontally, and the camera to a piece of board four to eight feet long, in such a manner that a line drawn through the axis of the tube of the microscope will fall exactly in the centre of the ground-glass of the camera.

It is well to mark the centre of the ground-glass with two short lines crossing each other at right angles, which may be drawn with a lead-pencil or with a diamond. These are to guide the operator in centring the field which he proposes to photograph. Such a camera as has been recommended, with the bellows extended, is about two feet long; the stand in a horizontal position will be fifteen to eighteen inches long. If these are mounted on a board four feet long, the microscope being placed six inches from one extremity and the camera flush with the other extremity, we shall have a very convenient and portable apparatus, which will do very well to begin with, and which may be *stretched* at any time by simply obtaining a longer board, placing the microscope and camera as before, and introducing a light-proof box or cylinder to couple the two parts of the apparatus.

The microscope must be fastened to the board in a firm and substantial manner, but in such a way that it may readily be detached; for this arrangement does not conveniently admit of the application of the eye to the eye-piece of the microscope for the purpose of searching a suitable field and focusing. It will therefore be found convenient in practice to detach the microscope for this purpose, and to place it in position on the board when a field has been selected. For this reason, the attachment should be by a clamp which can be quickly fastened, and that without interfering with the adjustment.

The connection between the camera and the microscope must be light-proof.

The operator will not be able to reach the adjustment screws for the purpose of focusing upon the screen when the bellows of the camera are extended to the full length, and the following expedient, therefore, is recommended to meet this difficulty: —

A drum of wood or metal two or three inches in diameter and half an inch thick is attached to the milled head of the coarse adjustment on one side of the instrument. This has raised margins, and serves to carry a cord which passes around it with one or two turns. One extremity of this cord passes through a hole in the board to which the instrument is attached, and supports a weight. The other extremity also passes through a hole in the support and around a pulley which changes its direction towards the screen end of the appa-

ratus. Here, within easy reach of the operator's fingers, it is fastened to a spool, around which it passes several times. This spool is made to revolve with sufficient friction to resist the action of the weight, and by means of it the operator is able to move the adjustment screw with great facility and precision.

The weight should be just sufficient to move the adjustment screw, and it will be found that a small bag containing shot (the proper quantity to be ascertained by experiment) will answer the purpose very well. Attach a metal hook to the bag of shot, and a ring to the end of the cord, so that the weight can be conveniently detached when the apparatus is not in use.

The diameter of the spool by which the movement for focusing is effected should be considerably less than that of its elevated margin, so that the operator may have the advantage of leverage in raising the weight. One inch for the axis, and two and a half to three inches for the margin, will be a proper size. The spool is to be turned out of wood or metal, and is fastened to the support by means of a single screw, around which it revolves. The necessary resistance to its revolution from the pull of the weight is effected by simple friction; or, better still, by the introduction of a coiled spring between the upper extremity and the board to which it is attached.

The fine adjustment is to be brought within reach in the same way; but this is more easily moved, and a simple groove around the milled

head will answer in place of the extra drum recommended for the coarse adjustment. Indeed, this drum may be dispensed with in the case of the coarse adjustment, if the head of the screw is of good size and the movement easy. For the fine adjustment, the cord used may be a well-waxed linen thread, and for the coarse adjustment, a piece of fish-line will be suitable.

This focusing apparatus must be attached in a workmanlike manner, and especial care must be taken in the placing of the pulleys by which the direction of the cords is changed after they pass through the board supporting the apparatus.

If the photographic apparatus is to be placed upon an ordinary table, it should be provided with legs placed at the four corners, and it is desirable that these be of such length as to bring the centre of the screen on a level with the operator's eye when he stands erect; for it is more convenient and less fatiguing to work in an erect position.

It is evident that this arrangement can be extended to any desired extent, by simply using a longer board to support the apparatus, and by introducing a light-proof coupling to connect the tube of the microscope with the camera.

The object of increasing the distance between the screen and the microscope is to give greater amplification with the same objective.

It is always desirable to obtain the amplification required with the lowest power possible, for the reason that

low powers have greater penetration. If, therefore, we can secure satisfactory definition with a one-inch objective at a distance of eight or ten feet, the result will be better than that obtained by the use of a higher power at less distance.

When the eye-piece is used in combination with the objective for the purpose of obtaining greater amplification, the screen cannot be removed to any great distance from the instrument, *for the eye-piece magnifies any imperfections in the correction of the objective, and introduces additional imperfections of its own,* which interfere with the definition of the projected image. For this reason, the best results are obtained by removing the eye-piece. The limit beyond which no increase of amplification, by increasing the distance of the screen, can be obtained without a sacrifice of definition, differs for different objectives, and depends partly upon the perfection of the objective as an optical instrument, and partly upon the fact that in ordinary objectives the corrections are made to give the best results at a definite point, namely, at the usual position of the eye-piece.

A series of objectives made especially for photography, and corrected to give the best possible definition at a distance of eight or ten feet from the screen, would possess some advantage over those not made for this particular work.

Lenses provided with cover-correction admit of adjustment, within certain limits, for increased distance; but it is not easy to make this adjustment when the cover-correction is out of reach, and

many of the best homogeneous immersion objectives are unprovided with cover-correction.

When such an apparatus as we have described is used in a room from which the light is not excluded, it will be necessary to shut off the light from in front of the objective, with the exception of that portion which is required for the illumination of the field. This is especially necessary when light reflected from the sky is used, and the time of exposure is comparatively long. A movable cover of wood or pasteboard blackened on the inside and made to fit over the microscope, may be used for this purpose. A circular aperture at the outer end admits the light to the object, and this may carry a condensing lens, if desired, when there is an achromatic condenser upon the sub-stage. If no achromatic condenser is used, this aperture should be very near the stage of the microscope, and diaphragms are employed to shut off all light except that required for the illumination of the field to be photographed.

It is evident that no modification of the apparatus described is required, if the intention is to use lamplight, except that the support should project in front of the microscope a sufficient distance to make room for the lamp. In this case, also, the cover intended to shut out diffused light from in front of the objective could be dispensed with, and a simple screen substituted for it, having an aperture at the proper level, into which the bull's-eye condenser could be introduced. A heliostat

might also be used, by placing the apparatus in a window facing the south.

The main advantage of this simple apparatus is that it is portable, and can be put aside when not in use, and consequently does not require a special room fitted up as an operating-room. When, however, it is practicable to devote a small room to the purpose, the arrangement next to be described possesses decided advantages.

V. OPERATING-ROOM AND FIXTURES.

When a room is available for the purpose, matters are very much simplified by making this the camera, inside of which the operator works. In this case, the room is darkened, and no actinic light is admitted, except that which comes through a properly located aperture to the achromatic condenser, or directly to the object if no condenser is employed.

This arrangement enables the operator to stand directly before the microscope — fastened upon a support in a horizontal position — for the purpose of selecting a field, and then to project and focus the image upon the ground-glass screen. This has a separate support, and is not necessarily coupled with the microscope by a light-proof connection. *When a heliostat is to be used,* the optical axis of the microscope must be on the meridian, and in a perfectly horizontal position.

The supporting shelf should be placed in a

window facing due south, and at such height that the eye of the operator is on a level with the eye-piece of the instrument when he stands erect.

A shelf outside the window supports the heliostat; and it is essential that this be accessible, for the purpose of adjusting the instrument, and removing it, for safety, when the operator has ceased working for the day.

Some little difficulty arises in regard to the placing and adjusting of the heliostat when this has to be managed through the window, and it is desirable to have an outside balcony or platform by which the operator can conveniently get at it from without. This is not essential, however; and by a little ingenuity the heliostat-shelf and window-fixtures can be so arranged that the instrument can be passed through a shutter and adjusted in its position on the shelf outside. In this case, the microscope should not be placed near the centre of the window, but as far as possible to the left, so as to leave room on the right for a shutter through which the operator leans to manipulate the heliostat. The upper sash of the window must be removed, and it is as well to remove both sashes and to close the window by a substantial and well-joined frame, which serves to exclude light and to support the shelf inside for the microscope, and outside for the heliostat. The right half of this frame carries the shutter which gives access to the heliostat. The heliostat governor (Woodward's) is attached to the frame in such a position that the axis

of the tube for admission of light coincides with the optical axis of the microscope.

The proper position of the heliostat-shelf is ascertained by adjusting the instrument in accordance with the printed directions accompanying it, and then placing it at such an elevation that, when it is in the same meridional plane with the microscope, the light from the mirror falls in the tube containing the eight-inch lens (in the Woodward governor), and is well centred upon the achromatic condenser of the microscope.

The operating-room need not be more than five or six feet wide by ten or twelve deep. The photographic manipulations may also be conducted in this room, but it is more convenient to have another small room, or closet, for this purpose. If there be a second window in the operating-room, this must also be carefully closed, to exclude actinic light; but sufficient non-actinic light may be admitted through one or two panes to enable the operator conveniently to find his way about the room. This is accomplished by substituting ruby glass for the common window-glass; or more economically, by fastening two thicknesses of good buff envelope-paper over the panes.

The admirable work of Surgeon Woodward, with which most microscopists are familiar, was done at the Army Medical Museum, Washington, D. C., in a room arranged as above described. Dr. Woodward has his ground-glass screen supported upon a small but substantial table, which moves upon rollers, and can be readily placed wherever desired.

The screen is adjustable in a perpendicular direction within the limits required. The arrangement for focusing consists of a pulley two and a half or three inches in diameter, which is fastened to the shelf which supports the microscope, in the same plane as the head of the fine-adjustment screw. A cord connects the two, so that the fine adjustment is controlled by moving the large pulley. The axis of this pulley projects towards the screen, and is square at the extremity for the reception of a key — like a clock-key — by which the pulley is operated. This key is attached to the end of a rod jointed like a fish-pole, which gives the operator control of the fine adjustment at any required distance from the screen.

Dr. Woodward has his microscope placed at such a height that the eye-piece is on a level with the eye of the operator when he is in a sitting position. The present writer, as already stated, prefers a standing position. This elevation of the instrument is also necessary when the screen is arranged in the manner about to be described, and the author's contrivance for focusing is adopted.

Instead of supporting the screen from the floor, the writer prefers to have it suspended from above, and movable in a right line corresponding in direction with the axis of the microscope. The focusing arrangement is essentially that already described (p. 51), but it is applied to the fine adjustment only, as the operator, standing at the microscope, can see the image upon the screen, and can focus roughly from this point. The cord, passing

around the grooved head of the fine-adjustment screw, is directed by pulleys attached to the framework *above the microscope*, and the spool or key by which movement is effected is attached to the wooden frame of the movable screen. This arrangement is more convenient than is that of the long-jointed rod, and the control of the fine adjustment is perfect at any position of the screen. Moreover, the focusing appliance is always ready, and at the same time is out of the way, as the cord passes from the microscope to the screen above the level of the operator's head. The advantage of having the floor clear in a small dark room will be appreciated by those who have done much work in such a locality. A smooth piece of two by four scantling, of proper length, will answer very well to support the movable screen.

When the design is to work *without a heliostat*, as is strongly recommended to beginners, it is not essential that a window having a southern exposure be selected, and the window-fixtures are simplified to a shelf for the microscope and some contrivance for excluding light, except such as comes through a small aperture opposite the opening in the stage of the instrument.

Unless the operating-room is in an elevated position, and the microscope, placed horizontally, looks out upon clear blue sky, it will be necessary to incline the instrument slightly, in the position of a telescope. It must be firmly fixed to its shelf in this position, and the elevation should be no greater than is absolutely required to effect the

object in view, as this change from a horizontal position is made at the sacrifice of some convenience as regards working.

It is evident that the scantling supporting the movable screen must have exactly the same inclination as that given to the microscope, so that the centre of the screen, in any position, may correspond with the optical axis of the instrument. This being the case, the operator can no longer stand erect before the screen when it is removed to its most distant point. In practice, it will be found most convenient to place the shelf upon which the microscope is supported at a greater elevation than when the instrument is in a horizontal position, in such a manner that the operator may still stand erect, or nearly so, when focusing upon the screen at its most distant point. This makes a little platform necessary just in front of the microscope, upon which the operator stands when working with the instrument.

The views obtained in a dark room through an instrument mounted in this way, either with or without an achromatic condenser (without heliostat), are so satisfactory, that it is worth while for one who does much work with the microscope to devote a room to this purpose, even if he does not propose to make photo-micrographs.

In many of the laboratories devoted to instruction in biology, histology, or pathology, the students may be seen seated before their microscopes at work under conditions as to illumination which fall very far short of the ideal illumination obtained

by such an arrangement as has been described. Light comes from all directions, and is reflected on the object from the white marble slab of the working-table as well as from the mirror. The polished stage of the microscope, especially with low powers, also reflects rays into the objective, and diffused or reflected light enters the eye-piece, and also the pupil of the observer's eye, to add to the confusion of the image. It is for this reason that many persons prefer to work by lamplight. The source of light being under control, these difficulties are avoided, with a decided gain of definition. But the quality of the light reflected from the sky is unsurpassed; and not only is the best definition possible, with the optical apparatus employed, obtained by this means, but the light is more agreeable and less injurious to the eye of the microscopist than is lamplight.

When a heliostat is used, the brief time of exposure makes it a simple matter to exclude extraneous light from the sensitized plate, and the dark room itself serves perfectly well as a camera. But without a heliostat, when the exposure is sometimes ten minutes or more, an extremely small amount of diffused actinic light in the room is sufficient to ruin the very sensitive dry plate. The operator would find it irksome to remain a prisoner in the dark room during these long exposures, but he cannot safely open a door for the purpose of leaving it, nor can he make use of a lamp for the purpose of reading, or of attending to other matters. For this reason it is desirable to have

a light-proof connection between the screen and the microscope during the time of exposure.

The arrangement of the writer's operating-room is as follows: —

There are two windows. The one in which the microscope is placed is entirely closed by means of tongued and grooved boards nailed to the sash inside. A metal tube an inch and a half in diameter is introduced into a circular opening in this partition, and this may be moved in and out for the purpose of making a connection with the substage of the microscope when this is adjusted for work. The effect of this arrangement is to exclude all light from the room except that which is admitted to the object through a diaphragm of proper aperture, or through the achromatic condenser, if one is used. The glass in the other window is covered with two thicknesses of heavy yellow envelope-paper. This admits enough non-actinic light to enable the operator to work in the room.

The camera recommended in Section III., which has a bellows capable of being extended to two feet, is suspended from a two by four scantling, one end of which is supported on the window-casing above the microscope, and the other end against the opposite wall (at a lower level, to secure the necessary inclination).

When working with the screen at a distance of two feet from the eye-piece of the microscope, this camera is sufficient to exclude extraneous light from the plate. At greater distances, the connec-

tion is made by means of a pyramidal-shaped bellows, which is suspended in the same manner as the camera is, and is joined to it by its larger extremity. A less expensive coupling may be substituted for this, and indeed any ingenious person will easily manage this matter by means of oblong or conical sections of tin or pasteboard *blackened on the inside.*

This placing a camera within a darkened room may seem an unnecessary precaution; but the slightest leakage of light through the plate-holder or the camera-box and connections would be a serious matter in such long exposures as are required when no heliostat is used.

In the apparatus recommended for use in a room not darkened (Section IV.), a box was to be placed over the microscope before making the exposure, to prevent diffused light from entering the objective in front of the stage. This will not be necessary when the room is darkened, and when the exposures are short, with low powers. And if the operator has no occasion to leave the room during an exposure, the light-proof coupling between the screen and the microscope may be dispensed with.

VI. PROJECTION OF IMAGE, AND FOCUSING UPON THE SCREEN.

We will suppose that the beginner has a dark room fitted up as described in the last section, and is ready to commence work. Having selected an object and placed it upon the stage of the microscope, he stands up before the instrument and looks over the preparation carefully, for the purpose of finding the best field to photograph. When he has decided upon this, and has adjusted his cover-correction — if the objective is provided with correction for cover — and his achromatic condenser, — if he is using one, — to secure the best possible definition and illumination, he removes the eye-piece. He then introduces a little cylinder of black paper or cardboard (dead black) into the tube of the microscope, to prevent reflection from the inner surface of the tube. The screen is next placed at such a distance as seems best, and the image is roughly focused upon it from the microscope, by means of the coarse adjustment. It will be remembered that the objective is always moved forward for this purpose, and that the greater the distance of the screen, the nearer the objective must come to the object.

The operator now steps to the screen and carefully focuses the image upon the ground-glass by means of the fine adjustment. The room must

be quite dark when this focusing is done, or light must be excluded from behind the screen by means of a black cloth thrown over the head, as in portrait photography. A very good plan is to have a shutter or curtain, by which all light is excluded from the window, covered with yellow paper. Or over the scantling which supports the camera a strip of cotton-velvet may be thrown in such a manner as to fall at the sides of the screen and exclude the light during the operation of focusing.

It will be found that delicate details of difficult objects cannot be seen upon the ground-glass: for this reason, *it is best to focus upon the aerial image in the position which will be occupied by the sensitive plate.* A focusing-glass is required for this purpose, and a piece of plain glass is substituted for the ground-glass of the camera.

As the ground-glass is also required for the rough focusing of the image from the microscope, it is well to have two swinging shutters, an external one containing the ground-glass, and an internal one carrying the plain-glass plate. When the focusing is completed, these shutters are swung back to make place for the plate-holder. As ordinary cameras are not provided with two swinging shutters, the ground-glass may be held in a frame smaller than the frame which holds the plain glass, and this can be placed in position and retained by a button, or removed entirely, when not required.

The light-proof connection between the screen

and the microscope must be arranged before the final focusing, as any disturbance of the apparatus is likely to disarrange the adjustment.

VII. MEASURING UPON THE SCREEN.

The facility with which microscopic objects can be measured when projected upon a screen, in the manner described, or from the photographic imprint of the image, is one of the advantages of this method.

To ascertain the real dimensions of the object from the measurements made of the enlarged image, it is, of course, necessary to know the exact amplification. This is ascertained by projecting upon the screen the lines ruled upon a stage-micrometer. If great accuracy is desired, the operator will do well to obtain one of the stage-micrometer slides made by Professor W. A. Rogers, of Cambridge, Mass.

Micrometer slides are ruled in fractions of an inch or of a *centimetre*. The usual unit of measurement for the smallest microscopic objects is the micromillimetre, for which the Greek letter μ serves as a symbol. The micromillimetre is the one-thousandth part of a millimetre — *i. e.* 1,000 $\mu = 1$ mm. —, which is equal to the one-twenty-five-thousandth part of an inch. The English micrometers are ruled in one hundredths and one thousandths of an inch.

If we project upon the screen the lines on a

stage-micrometer, ruled in fractions of an inch, and find that the lines one-hundredth of an inch apart are separated from each other by exactly one inch, it is evident that the amplification effected by the optical combination used, with the screen at this distance, is one hundred diameters. If the lines one thousandth of an inch apart on the micrometer are one inch apart on the screen, the magnifying power is one thousand diameters, etc.

The exact amplification of every photo-micrograph made should be recorded, and it is desirable that the magnifying power employed be expressed in hundreds or even fractions of one hundred, as 500, 250, 50, 20, etc. For this purpose, and to save the labor of making measurements whenever a different objective is employed, or the position of the screen changed, the beginner is advised, before making any attempt to photograph, to mark upon the scantling from which the camera is suspended, the position of the screen with an amplification of 100, 200, 300, etc. (or fractions of 100 with lower powers), for each objective or optical combination which he proposes to use.

For example, if a one-inch, a one-fifth-inch, and an amplifier are the optical appliances to be used, the operator would first determine the position of the screen for the one-inch alone, marking by a perpendicular line the exact location of the screen when the one-hundredth-inch lines are one fourth inch apart, — twenty-five diameters; one half inch apart, — fifty diameters; one inch apart, — one hundred diameters, etc.

The writer is in the habit of making this record upon the right-hand side of the scantling upon which the camera slides, as follows, —

$$\frac{100}{1 \text{ in.}} \quad \frac{200}{\frac{1}{2} \text{ in.}} \quad \frac{50}{1 \text{ in.}} \quad \text{etc.}$$

After making this determination for the one-inch alone, make it for the same lens in combination with the amplifier; then for the one-fifth-inch; and finally, for this and the amplifier together.

The exact point is fixed in each instance by moving the screen forward or back until the lines are at the proper distance from each other, to give the desired amplification. This is determined most conveniently by a pair of dividers, which are adjusted from a scale of inches. Suppose, for example, that the points of the dividers are adjusted to one inch apart. The screen is now moved to such a position that four of the one-hundredth-inch spaces (five lines), fall between the points of the dividers. The amplification will evidently be twenty-five diameters. Now move the screen farther back until two of these spaces fall between the points of the dividers, and we have fifty diameters, etc. It will be necessary carefully to focus the lines for each determination, and to place the points of the dividers precisely in the centre of the dividing lines.

Having made this determination once, there will be no further trouble about measuring. It must be remembered, however, that the condi-

tions must be identical as regards the position of the optical appliances, or a certain amount of error will result. Thus, if the amplifier is moved to a different position in the tube, or the cover-correction of an objective is changed, the magnifying power of the combination will be slightly altered.

VIII. DIAPHRAGMS IN THE PLATE-HOLDER.

A DIAPHRAGM with a circular or oval aperture introduced into the plate-holder, in front of the dry plate, is a convenient way of giving a good form and definite boundary to the field. The light is arrested by this diaphragm, so that the part covered is transparent in the negative, while in the positive the picture appears framed in black. (See Plate XI. Fig. 2.) The effect obtained in this way is especially good when the negative is to be used for printing positives on glass for projection.

Silk thread or horsehair may be stretched before the plate for the purpose of printing fine black lines upon the positives. The writer sometimes resorts to this device for the purpose of showing the amplification upon the screen when the glass positives are to be used to illustrate popular lectures. Black cardboard may be used for diaphragms in front of the plate, and lines may be drawn by attaching black thread or horsehair to this with sealing-wax.

A square diaphragm, covering one half the plate, may be used for the purpose of bringing two different pictures in juxtaposition on the same plate. The first exposure is made with one half the plate uncovered; the second, after changing the diaphragm to the previously uncovered side. By this means, different objects which it is desired to compare are brought together and printed from the same negative, — *e. g.* the blood-corpuscles of man and of the dog; or the same object may be taken on the two sides of the plate with a different amplification. This method is useful also for comparing the performance of different objectives; for the conditions being identical as to time of exposure, development of plate, printing of positive, etc., differences of definition must be ascribed entirely to the optical part of the apparatus.

IX. EXPOSURE OF PLATE.

No definite rules can be given as to time of exposure, and the beginner must be prepared to expend considerable material in learning to estimate the amount of light by an inspection of the screen when all is ready for the exposure.

The elements upon which the result depends, as seen in the illuminated field upon the ground-glass plate, are: the source of illumination, — sunlight or artificial light; the use of a heliostat, — direct light, or light reflected from the sky; the

position of the achromatic condenser, when one is employed; the power and angle of aperture of the objective; the use, or otherwise, of an amplifier; the distance of the screen.

When direct sunlight, reflected by a heliostat, is employed, the exposure with the extremely sensitive dry plates is very short, and with low powers it will be necessary to resort to the use of a mechanical contrivance by which the plate is exposed for the fraction of a second. This consists of a "drop-screen" placed between the blue-cell and the achromatic condenser, which has an adjustable rectangular opening in the middle line, which admits the light to the plate during the time occupied in passing in front of the line of light.

In general terms it may be said that, *The actinic power of the light is less when the sun is low in the heavens, and when the atmosphere is hazy.*

Light is always lost by the use of reflecting surfaces or refracting media; consequently the more lenses it has to pass through on its way to the screen, the weaker it will be, independently of the attenuation due to divergence of the rays.

Low powers admit more light, because of the greater diameter of the front lens, which affords a greater area for admission of light. In lenses of the same magnifying power, the comparative amount of light admitted depends upon the angle of aperture. An immersion-lens admits more light than a dry-lens of the same angle; a *homogeneous* immersion objective, more light than one used with

an immersion fluid of a lower refractive index, *e. g.* water.

The light is attenuated after emerging from the objective, by the divergence of the rays. The angle of divergence depends upon the magnifying power of the objective, and the attenuation of light will depend upon this angle and the distance of the screen on which the enlarged image is projected. This attenuation increases as the square of the distance, while the magnifying power — in diameters — increases in simple ratio with the distance; *e. g.* if the magnifying power is one hundred diameters at a distance of two feet, it will be two hundred diameters at four feet, and the time of exposure will be four times as great.

While no precise rules can be given as to time of exposure, owing to the number of elements upon which this depends, some data may be given which will possibly prove useful as hints to beginners.

Working with a heliostat in southern latitudes (Havana and New Orleans), the time of exposure for wet plates, when the amplification was about fifteen hundred diameters, and the lens employed Zeiss's one-eighteenth-inch homogeneous immersion, was from eight to fifteen seconds. (See photographs of blood-corpuscles, Plate VI.)

With Beck's one-fifth-inch, and an amplification of four hundred diameters, the exposure was almost instantaneous.

At San Francisco without a heliostat, — re-

flected light from the sky, — and using Eastman's instantaneous dry plates, the time of exposure with a one-inch objective (Collins's) is from one to two minutes when the amplification is fifty diameters. At the same place and under the same conditions, a photograph of Bacteria (*B. rubescens*, Ray Lankester), in which the amplification was six hundred diameters, and the objective used was Zeiss's one-eighteenth-inch homogeneous immersion, the time required was twenty-five minutes.

These long exposures would be impossible with wet plates; but a dry plate may be exposed for any length of time required, and, as already stated in the section on Light, any image that makes a sufficiently distinct impression on the retina to enable the operator to focus it properly, may be photographed.

Professor C. H. Kain has been kind enough to send me some of his photographs made by lamp-light.[1] The notes upon these show that the time of exposure, when the amplification was about twenty-five diameters, was from three to six minutes.

In an *under-exposed* photograph of *Arachnoidiscus ornatus*, made with Wales's one-tenth-inch objective and A. eye-piece, — amplification about three hundred and fifty diameters, — with illumination by student's-lamp, bull's-eye condenser, and achromatic condenser (Carbutt's J. C. B. plate), the time of exposure was ten minutes.

[1] See his paper in the Amer. Month. Micr. Journal, Vol. III. No. 4, p. 71.

The time of exposure depends upon the nature, and especially upon the color, of the object, as well as upon the source of light and the optical apparatus used. Transparent objects, in which the details are not sharply marked, require comparatively little time, and if overtimed, all delicate markings or details of structure are lost in such objects. They should therefore receive a short exposure and long development, and may require intensification.

On the other hand, opaque objects, or such as are practically opaque for the actinic rays, namely, those of a deep red, yellow, orange, or brown color, will entirely arrest the chemical rays, and no details of structure can be obtained, no matter how long the exposure.

For this reason, many objects which appear very beautiful under the microscope, such as the brown septate spores of some of the microscopic fungi (*Coniomycetes*), pollen grains of large size and of a deep yellow color, deeply stained (carmine) sections of animal tissues, etc., are quite unsuitable for photography.

These colors, however, are used in feeble degree of intensity, to give photographic definition or contrast to transparent objects, and to bring out structural details, by virtue of the special affinity which certain staining agents have for certain histological elements of animal and vegetable tissues. Violet is theoretically transparent for the actinic rays. The colors from the violet end of the spectrum, however, are better adapted for the staining

of objects which are to be photographed by transmitted light than are those from the red end of the spectrum.

In making photo-micrographs, the effort of the operator should be to produce negatives having good printing qualities, from which well-defined silver-prints can be made, and in which the background shall be as light as possible. The object should be sharply drawn, showing all details of structure possible.

When a good field has been found and carefully focused upon the screen, it will generally be necessary to make a number of exposures, in order to obtain the best possible photographic result. Each plate is given a little more or less time than the preceding one, as seems most desirable. If the operator is convinced by a careful comparison of the negatives that one of them is as good, photographically, as can be made, it is a good plan to make another exposure of the same duration, so that he may have a duplicate in case of injury to one of the negatives. All the inferior negatives may as well be rejected at once, for it is hardly worth while to retain poor negatives when we have better ones of the same object; and when we have a field upon the screen from which a desirable photo-micrograph may be made, it is poor economy to spare plates until the best possible result has been attained.

Over-exposure produces a feeble image, which appears quickly when the plate is placed in the developing solution, and in which the details are

lost or obscured. If we look through an over-exposed negative towards a strong light, as in front of an open window, we may see the image quite distinctly; whereas the positive picture obtained by placing a piece of black cloth or pasteboard behind the negative will scarcely be seen at all, or will appear weak and indistinct.

Under-exposure is recognized in developing by the slowness with which the image appears, and the absence of any photographic imprint from the less transparent or more deeply colored parts of the field. No amount of developing or intensification will give the necessary strength, even to the background, when the plate has been considerably under-exposed, and after fixing, the shadows in the picture, viewed as a positive, will be entirely transparent, whereas in the over-exposed plate they were grayish or foggy.

To get the best general result, it will often be necessary to give an exposure which is longer or shorter than would be desirable for some parts of the picture. Thus, if the object be a very thin diatom having delicate markings, it will be necessary to give a brief exposure, for the purpose of preserving these, and the background will consequently be weaker than would be desirable. The result will be that in the positive, the outlines of the object will not be in strong contrast with the ground of the field in which it appears.

In single objects having regular outlines, — *e. g.* diatoms, — this may be readily remedied by filling in the background by hand with an opaque

paint, or by the use of a paper diaphragm in printing.

Again, a marked difference in the transparency or color of different portions of the field makes it impossible to give the time required to bring out the details in certain places without over-timing the background, and the more transparent parts of the picture. Photographers who are not familiar with the difficulties peculiar to photo-micrography are not fair critics of pictures of this kind, which are not to be judged as photographs, but as photo-micrographs; and it will not do to sacrifice microscopical details for the purpose of making an ideal photograph.

X. DEVELOPMENT OF PLATE.

BEGINNERS are advised to use the ferrous-oxalate developer, and there seems to be no good reason why they should change to any other, after they have learned the use of this. The formula is simple, the developer works in a satisfactory manner, and, so far as the writer can judge, the results are as good as those obtained by any other method.

Formula.

Oxalate of Potash, a saturated solution.
Protosulphate of Iron, a saturated solution.
Filter each solution, and keep in separate bottles.

Just before using, add one part of the iron solution to six or seven parts of the oxalate. The

resulting solution of oxalate of iron should be of a rich ruby color and free from precipitate.

It is best to mix the developer in a wide-mouthed glass vessel, so that it may be poured over the plate at a single sweep. A small tumbler or a wide-mouthed bottle having a capacity of from two to four ounces will answer very well. If the smallest-sized plates ($2\frac{3}{4}\times3\frac{1}{4}$) are used, an ounce of the developer will be a sufficient quantity to mix at one time, and this may be used to develop several plates in succession. The iron solution must always be added to the oxalate. If it be in too small quantity, the development will be slow and imperfect; if in too great quantity, a yellow precipitate will be deposited upon the plate.

The developing-tray may be of hard rubber, porcelain, or glass. A cheap oblong glass dish with flat bottom answers very well for quarter-size plates ($3\frac{1}{4}\times4\frac{1}{4}$). It must be kept scrupulously clean. In general it may be said that in photographic operations every solution used should have a receptacle which is devoted to its sole use.

The developer must thoroughly cover the plate, and if any air-bubbles are to be seen which cannot be removed by gently tipping the developing-tray forward and back, they should be removed with a soft camel's-hair brush.

The image does not appear as quickly upon a dry plate as upon a wet one, as some little time is required for the solution to penetrate the film;

and when the light has been feeble and the time of exposure long, the image often seems to be quite superficial.

The development should be continued until the image is seen in all its details from the back of the plate. If it will not develop entirely through the gelatine film, it is because the exposure has been too short; and the superficial image seen upon the front of the plate, although apparently well timed, will be very faint after the plate has come from the hyposulphite bath.

The ferrous-oxalate developer, if not too strong, does not injure the plate, even if it be left in the solution for fifteen or twenty minutes, and *in making photo-micrographs it is generally best to give rather a short exposure, and then to develop the plate to the fullest extent.* The developer does not act upon that portion of the plate unaffected by light, and the effect of this long development is simply to intensify the image and bring out all the finer details. The writer is in the habit of leaving his plate in the developing-tray while he attends to the arrangement and focusing of another object, and often several exposures are made, and the plates placed in separate developing solutions, before the first exposed is considered fully developed.

For other methods of development the reader is referred to manuals of photography.

XI. FIXING, INTENSIFICATION, AND PRESERVATION OF NEGATIVE.

When the development is completed, the plate is to be washed and placed in the "fixing bath," which consists of a strong solution of hyposulphite of soda. The effect of this is to render the film transparent in those parts not acted upon by the light, which, previous to this operation, were opaque and of a grayish-white color. The plate should remain in the "hypo" just long enough to clear it up, and should then be thoroughly washed.

In warm climates, and during the summer months in northern latitudes, the dry plates of some manufacturers require to be washed with iced water, to prevent the film from peeling off—"frilling." At San Francisco, where the summer temperature rarely exceeds 70° Fahr., I have not found it necessary to resort to the use of iced water, and the manufacturer (Eastman) of the plates which I have been using advertises plates especially prepared for use in tropical climates, which he claims will not frill when washed with water at the ordinary temperature of that found in the tropics.

A strong solution of common alum is sometimes used before or after fixing, to harden the film and cause it to dry more quickly. The writer has not found it necessary to use it in connection with the

ferrous-oxalate developer. It may be that the long time during which the plates remain in the developer has a tendency to harden the gelatine film and prevent frilling.

Intensification. A moderate degree of intensification may be effected by means of a saturated solution of bichloride of mercury (corrosive sublimate) and a ten-per-cent solution of strong ammonia.

The plate is first flooded with the solution of bichloride, until, viewed from the back, the image appears uniformly gray; it is then thoroughly washed, and finally is placed in a bath containing the ammonia (ten per cent) until it is blackened.

The following method of intensification is recommended by Eastman for his dry-plates: —

"Prepare saturated solutions of iodide of potassium, bichloride of mercury, and hyposulphite of soda. Pour the mercury into the iodide until it retains a red color after shaking. Now add enough of the hypo solution to dissolve the red precipitate. If the solution refuses to intensify, there is too much hypo in it; therefore, prepare another portion of the mercury-iodide solution, and add just enough to the intensifier already made to clear it up, instead of the hypo. This solution will keep.

"If extra intensity is desired, add a few drops of a fifteen-grain solution of bromide of potassium to each ounce of developer before applying it to the plate." (Eastman.)

The dark room in which the plate is developed may be that in which the exposure was made, if all

white light has been excluded from it; but if there is light enough in this room to affect the plate in the slightest degree, it will be necessary to remove it from the plate-holder and commence the development in a dark closet, or in a room specially devoted to photographic manipulations. After the plate has been in the developer a short time, it is no longer injured by a small amount of light, and the operations of fixing and intensifying may be conducted in a light room if desired.

A liberal supply of water will be required for washing the plate after it comes from the different solutions used, and a running stream with free escape for waste-water is very desirable. A stationary washstand with the usual fixtures, in one corner of the operating-room, or in an adjoining closet, will answer the purpose admirably.

If the operating-room has one window covered with yellow envelope-paper, as has been recommended, it will be well lighted for the photographic manipulations; and if it is found that enough actinic light enters the room to injure the plate during the time occupied in removing it from the plate-holder and covering it with the developer, this part of the operation may be conducted in a dark closet, or in a corner of the room partitioned off for the purpose.

The negative should be carefully inspected after the final washing, and if it pass muster, is to be placed aside *to dry spontaneously*. A convenient arrangement for supporting the negatives while drying, is to nail a strip of board to the wall of

the operating-room, and to place a line of nails, in pairs, along the centre of this. These nails should be about two or three inches apart, and are only partially driven home. The negative is supported between them in an oblique direction, only the lower corner touching the board.

Negatives may be defective from any of the following named causes: —

Improper adjustment of the illuminating apparatus, causing the plate to be unevenly lighted; light from heliostat or achromatic condenser not well centred; or obscuring details from imperfect focusing of condenser.

Foggy appearance, due to the admission of extraneous light to the plate, — from beneath the object, to be remedied by diaphragms; from diffused light, or light reflected from the stage entering the objective, remedied by working in a dark room; from cracks or imperfect connections in the plate-holder and camera.

Circle or spot of light, due to reflection from interior of tube of microscope, to be remedied by cylinder of black paper; or from reflecting surface upon interior of camera or its connections with the microscope — remedied by blackening.

Imperfect definition, from use of inferior lenses; imperfect cover-correction; straining the lens, as to distance from screen, beyond what it will stand; improper adjustment of amplifier, if one is used, in the tube of the microscope; or imperfections introduced by eye-piece, if the attempt is made to use one.

Extraneous objects in the field, or objects in the background which are out of focus.

Movement of objects, in fluid mounts, during time of exposure.

Dirt upon the slide behind the object, upon the cover-glass, or upon any part of the optical apparatus.

Imperfect focusing; movement of apparatus during time of exposure, due to vibration of building caused by the wind, passing trains, etc.

Over-exposure produces weak and foggy image.

Under-exposure produces strong and clear shadows.

Under-development produces weak image and clear shadows.

Dust or air-bubbles on the plate produce transparent spots.

Hyposulphite of soda not well washed out causes an appearance of crystallization when the negative is dry.

Too much iron in the developer causes a yellow gritty precipitate upon the plate.

When a negative is defective from any of these causes, TRY AGAIN.

Owing to the many causes of failure and the difficulties of technique already pointed out, *a good negative is a valuable piece of property*, and should be carefully preserved.

Those which are to be retained, when thoroughly dry, are to be varnished with negative varnish. Mountfort's crystal varnish has a good reputation, and this or any other varnish recom-

mended by a practical photographer may be used.

A sufficient quantity of the varnish is poured upon the plate, held by one corner in a horizontal direction, and this is caused to flow over the whole surface in a uniform manner by tipping the plate. The surplus varnish is allowed to flow back into the bottle from one corner of the plate. The varnish dries quickly, and the plate should be placed upright until it has hardened.

Every negative preserved should be numbered by scratching the number through the film in one corner, or by means of a small paper label attached by mucilage.

The number of each negative, the date upon which it was made, the amplification, the objective used, and the nature of the object photographed, together with remarks which may prove useful in future operations, with reference to time of exposure, staining agent used, etc., should be recorded in a book kept for this purpose. The negatives may be conveniently stored in the paste-board boxes in which the dry-plates are received; the numbers of the negatives in each box being written upon a slip of paper attached to the front of the cover, for convenience in finding a particular negative when a series of boxes are piled one upon the other.

XII. PHOTOGRAPHING BY REFLECTED LIGHT.

In the section on Light, some remarks have been made on the subject of photographing opaque objects by reflected light. There are serious difficulties to be encountered, which interfere with the use of high powers for this purpose; but with comparatively low powers many interesting microscopic objects may be photographed which it would be quite impracticable to photograph by transmitted light. The light is thrown upon the object by means of a Lieberkühn, and the writer has found the best source of illumination to be sunlight reflected from the sky. Direct sunlight reflected from the mirror of a heliostat has not proved satisfactory.

Means must be provided for focusing the Lieberkühn separately from the objective, so that the best possible illumination of the object may be obtained. The writer's method of accomplishing this has been described in Section II. For this kind of work it is essential that the objective have the greatest possible penetrating power.

No light must enter the objective except that reflected from the object; and to secure good definition it will be necessary to use a diaphragm between the objective and the object. A very long exposure will be required. The head of a fly, magnified about twenty diameters — three-inch objective — required an exposure of twenty-five minutes.

The manner in which this object was mounted for photography will serve to illustrate the general method of manipulation.

First catch your *fly*. Kill it by compressing the thorax with forceps, and examine the head with a magnifying-glass, to see that it is uninjured and free from dust. If it is not perfect, renew the hunt. The dust may be removed by careful brushing with a camel's-hair brush. Select a fly with a white forehead, for this reflects the light better than black; place a minute drop of glue, or of Canada balsam, in the centre of a perfectly clean slide; decapitate the fly and attach the head to the slide by means of this; in like manner attach a circular piece of black cloth or paper — dead black — to the opposite side of the slide. This serves as a background, and must be of such size that all direct light is prevented from entering the objective, while as much light as possible passes outside of it to the Lieberkühn.

The object thus prepared is placed upon the stage of the microscope, and carefully centred in the optical axis of the instrument.

The slightest speck of dust upon the object or upon the surface of the glass slide in the field of view, reflects the light strongly, and mars the beauty of the picture.

XIII. POSITIVES UPON GLASS.

Positives on glass are used mainly for lantern projections. They are admirably adapted for the illustration of popular lectures, and are made with such facility that one who has a good series of negatives can always produce them at short notice. No camera is required, as the process by contact gives excellent results, especially with dry-plates.

The writer's method of manipulation is as follows: The negative is placed in the plate-holder with the film side to the back; the dry-plate is placed in contact with this film to the front; a piece of black cardboard of the same size is placed back of this; the plate-holder is then closed and held up in the proper position with reference to the light; the shutter is drawn quickly, to make the exposure, and quickly replaced after the proper interval, which will always be very brief.

A steady artificial light — gas or oil — may be used; but the writer prefers to use daylight, admitted to his operating-room through a small opening in one of the windows. The size of this opening is regulated by means of a sliding shutter, covering a single pane of glass when closed. A slit an inch or two wide will admit an abundance of light, when the exposure is made on the opposite side of a room ten or twelve feet wide. Even with this narrow opening, the time of ex-

posure is so brief that it is better to work on a cloudy day, or late in the afternoon, when the light is already too feeble for making negatives. The dry-plate must be introduced into and taken from the plate-holder in a room from which all actinic light is excluded. If the operating-room is sufficiently protected when the sliding-shutter is closed, this will answer the purpose; otherwise a dark closet will be required.

A small lantern, dark on three sides, and having the other side closed with ruby glass, will be required for manipulations conducted in a dark room from which all light is excluded.

The development of the positive and other photographic manipulations is conducted in exactly the same manner as in the case of a negative.

A very fair positive may be made on glass from a silver print — on paper — by the following method: If the silver print is mounted on a card, detach it by immersion in *hot* water for a few minutes; dry it thoroughly under pressure, so that it may be quite smooth; place a piece of plane-glass in the plate-holder, next to this the silver print, then a dry-plate with the film in contact with the printed image upon the paper, and finally a piece of black cardboard, as a non-reflecting background; close the plate-holder, and expose as directed for positives on glass.

The result, if the exposure is well timed, will be a very fair negative, from which, when finished, positives on glass may be printed.

The writer has thus been able to obtain negatives from silver prints made years ago, which were excellent, with the exception that the texture of the paper shows to some extent. It may be that the result would be better still if the paper upon which the silver print is, should be made transparent with oil or varnish.

Another hint, which may be useful to those who use a lantern for the illustration of popular or scientific lectures, has reference to the possibility of making outline drawings with a sharp-pointed instrument upon the gelatine coating of a dry-plate. The lines of the negative drawing must be clean-cut, and entirely through the film. The negative may be used in the lantern directly, or a positive may be printed from it on another plate by the method already described.

Those who desire to make their own silver prints are referred to manuals of photography for details as to the method of doing so. As already stated, the amateur photographer is advised to place his negatives in the hands of the nearest practical photographer, when he desires a small number of silver prints from them.

XIV. SELECTION AND PREPARATION OF OBJECTS FOR PHOTOGRAPHING.

Success in making photo-micrographs depends quite as much upon the selection of suitable objects and upon their proper preparation with

reference to photography, as upon the optical apparatus used and the technical skill of the operator as a microscopist and photographer.

In the present section, the writer proposes to indicate the kind of objects most suitable for making photo-micrographs, and the methods of preparation which have given him the best results.

In giving this account, special reference will be had to the objects which have furnished the illustrations for the present volume, and these will be taken up in the order in which they appear in the plates.

Micrococci. (Plate II. Fig. 2.)

These minute vegetable organisms require a high power for their detection. When properly stained, they may be photographed with a good one-tenth-inch objective; but a higher power is better. The writer has obtained his best results by the use of the one-eighteenth-inch homogeneous immersion objective of Zeiss.

It is well to adopt a standard amplification for each class of objects, so that they may be readily compared as to dimensions by a simple inspection of photo-micrographs, made at different times and places. The standard adopted by the writer for bacterial organisms, — *Bacteria*, — the class to which our Micrococci belong, is one thousand diameters. A less amplification than this will not show the smallest Micrococci in a satisfactory manner, and a greater is not necessary for a majority of the Bacteria.

The photographic method is especially adapted to the study of the Bacteria; and these minute organisms possess a peculiar interest at the present day, because of the important *rôle* which they have recently — within fifteen or twenty years — been shown to play in the economy of nature, as the cause of the putrefaction and fermentation of organic substances, and of certain infectious diseases, and because there is yet much to be learned about them. Bacteria of various forms and dimensions are found in vast numbers in animal or vegetable infusions exposed to the air; but in this case different species are commonly mingled together, and the Micrococci, especially, are rarely found unmixed with other forms. There are various methods of obtaining a "pure culture," but the limits of this work oblige the writer to confine his remarks to that adopted in the experiment which furnished the material for the photo-micrograph under consideration.

It is well known that a variety of bacterial organisms are constantly found in the mixed salivary secretions present in the healthy human mouth. A little saliva scraped from the surface of the tongue, dried upon a thin glass cover, and stained with a solution of aniline violet, will invariably be found to contain several different species.[1]

Among these is a micrococcus which does not present precisely the same appearance as that

[1] See paper by the present writer published in "Studies from the Biological Laboratory," Johns Hopkins University, Vol. II. No. 2, p. 157.

seen in the photo-micrograph, but which, nevertheless, is proved by experiment to be identical with it. If a little saliva containing these various organisms be injected beneath the skin of a rabbit, the animal dies within two or three days, and its blood is found to contain vast numbers of the micrococcus seen in Fig. 2, Plate II.[1] We have thus, by the expedient of introducing a fluid containing a variety of bacterial organisms into the body of a living animal, obtained a "pure culture" of a single organism, evidently because *the conditions were favorable for the multiplication of this one, and not for that of the others.*

Upon the same principle, we may obtain a pure culture if a drop of fluid containing different micro-organisms is added to a "culture-fluid" suited to the development of one only; or to a fluid maintained at a temperature which is favorable for one species and not for the others.

In the case under consideration, a minute quantity of blood from the veins of a rabbit just dead, as the result of the experimental inoculation referred to, was spread upon a thin glass cover and allowed to dry. It was then stained with a weak solution of iodine (iodine, grs. iii, potassic iodide, grs. v, distilled water, grs. 200). The peculiarity of this micrococcus, as it exists in the blood of a rabbit, is that it is surrounded by an aureole of transparent material which it is difficult to see in unstained preparations, because the refractive

[1] See paper by the writer in "Studies from the Biological Laboratory," l. c. p. 184.

index of this substance is nearly the same as that of the blood serum in which the organism is suspended. This substance is deeply stained by iodine, and the result is that in the photograph it appears as a well-marked aureole surrounding the Micrococci, and separating them from the dried blood serum which forms the background of the field.

The method of mounting bacterial organisms in general, for the purpose of photographing them, is essentially that employed in the instance given. A drop of the fluid containing them is spread out upon a very thin and perfectly clean glass cover. This is allowed to dry, and the Bacteria are thus attached to the cover in a very thin and tolerably uniform layer.

The aim of the operator in preparing unicellular organisms or vegetable and animal tissues for photography, should always be to secure a single layer of cells; for when the cells are piled upon each other, those in the background are necessarily out of focus, and interfere with the beauty of the picture.

The aniline colors are especially adapted for staining the Bacteria. Of these the methyl violet and aniline brown are the most generally useful. Other colors are useful for the differentiation of certain species, *e. g.*, the bacillus of tuberculosis described by Koch. A good solution of methyl violet for the purpose is found in the violet ink to be purchased for a trifle from almost any stationer. This should be filtered

before using. A drop of the staining fluid is placed upon the thin glass cover, to which the organisms to be stained are attached, and after a short interval is washed away with a gentle stream of distilled water, or by agitating the cover, held by forceps, in a glass of water. The Bacteria take the dye very quickly, and a few seconds will generally suffice to stain them deeply with one of the aniline colors.

Methyl violet is generally preferable, as the aniline brown stops out the light so completely as to destroy any details in the interior of the Bacteria, such as granules or endogenous spores.

Micrococci specifically different from those seen in the photo-micrograph under consideration may be found in urine which is undergoing the alkaline fermentation (*Micrococcus uræ*, Cohn). A variety of Bacteria may be found in the stagnant water of gutters and small ponds. They may be frequently detected as an iridescent film upon the surface of the water, and this is readily transferred to a thin glass cover by gently bringing the cover, held by forceps, in contact with the surface of the water.

In the same way Bacteria may be picked up from the surface of moist mud or from an exposed mucous membrane of man or of one of the lower animals. Bacteria may also be cultivated especially for the purpose by exposing a variety of organic infusions to the air. Floating germs fall into these, and different species develop in the different culture-fluids, according as one or the other finds the medium most suited for its development.

For preserving a pure culture of any particular species, the culture-flask and method of manipulation described by the writer in his paper already referred to, is recommended.

The thin glass cover, with the stained Bacteria attached, is mounted upon a slide by means of a circle of white-zinc cement, made upon a turn-table. The shallow cell made by this circle of cement is filled with camphor-water, weak carbolic-acid water, or simply distilled water.

Great care will have to be exercised in the selection of a field, and in focusing, in order to obtain a satisfactory picture.

Amœbæ. (Plate II. Fig. 1.)

Especial attention is called to the photo-micrograph of an amœba (Plate II. Fig. 1),[1] as it is a photograph from life of this interesting and much-talked-of creature, and because it illustrates the fact that transparent objects are the best suited for photography, inasmuch as they alone show interior details of structure in a satisfactory manner.

The interior details seen in this case, however, are not details of structure, except perhaps the cavity just above the dark-colored sphere, which is a vacuole. The sphere, which might be mistaken for a nucleus, is a unicellular alga of a bright red color (winter form of *Protococcus*, or so-called *Hæmatococcus*), and was taken in by the

[1] An accident to the negative has made it necessary to substitute another and inferior photo-micrograph of Amœba for the one described in the text.

creature at its last meal, which it was quietly digesting while sitting for its photograph. The granular material seen about this colored sphere is the partially digested remnant of former meals. The outline of the jelly-like mass, which is flattened out against the glass cover, is very distinctly seen, and below has a wavy appearance. On the left is a small object out of focus, probably a starch-grain, in contact with the amœba.

Transparent objects which have a different refractive index from that of the medium in which they are placed, do not usually require to be stained; for the increased photographic contrast which is obtained by staining destroys the natural appearance, and the picture no longer conveys the idea that the object is transparent. It is consequently brought nearer to the level of a woodcut, and, to a certain extent, loses its value as a photo-micrograph.

Amœbæ are found in ponds and ditches, and are commonly attached to the surface of decaying leaves, water plants, etc.

Unicellular Algæ.

The unicellular Algæ should be mounted for photography in very shallow cells, made by turning a circle of white-zinc cement upon a slide. Their color and natural appearance will be preserved in an aqueous medium, such as weak carbolic-acid water or camphor water. Unfortunately, photography cannot reproduce the rich ruby color of *Protococcus nivalis*, or the bright green of *Protococcus viridis*. The deeply colored

protoplasm of the former arrests light entirely, and we have in a positive print only a uniformly black mass with circular outline, surrounded by another line representing the cell wall, to represent the beautiful little ruby sphere with its more or less granular contents. The green color of *P. viridis* is better adapted for photography. Plate III. Fig. 1, represents one stage in the life history of the last-named species.

The unicellular Algæ are found in pond and ditch water, upon the surface of moist mud, damp walls and stones, etc. They are interesting microscopic objects, and suitable for photographing. *P. nivalis* grows upon the surface of the snow in high latitudes, and upon snow-clad peaks in more temperate regions. An amplification of from two to six hundred diameters will be required. For the lower power, Tolles's four-tenths-inch or Powell & Lealand's one-half-inch objective may be used; for the higher, a good one-fifth-inch lens.

Infusoria.

Many of the Infusoria may be successfully photographed, but it will be necessary to exercise great care in the preparation of slides for this purpose. Generally, but a single individual should be in the field of view, and this should be a perfect specimen; for it is difficult to obtain fields containing several individuals all in the same plane, and in order to show cilia, flagella, and interior details of structure, high powers and very careful focusing will be required.

Occasionally a living infusorium may be quiet long enough to have its photograph taken; but usually the Infusoria are in rapid motion, and it will be necessary to arrest this motion by means of some chemical agent fatal to their vitality. A weak solution of iodine does this very effectually, and at the same time stains the protoplasm a brownish color. A ciliated infusorium killed by adding a drop of this solution (iodine 1 gr., potassic iodide 2 grs., water 100 grs.) to a drop of the fluid in which it is swimming, remains for a time as if suddenly frozen, with its cilia rigid, and projecting, like rays, from the surface of the body. This is the favorable time for photographing the creature, as, later, it is liable to undergo changes which destroy the internal structure.

Another method is to place a drop of fluid containing the Infusoria in the centre of a clean glass slide, and to invert this over the mouth of a bottle containing a one-per-cent solution of osmic acid. A very brief time is sufficient to destroy the life of the Infusoria, which may then be selected under a low power and transferred to a drop of clean water. They must be mounted in the thinnest possible stratum of fluid, otherwise they are likely to change position while the exposure is being made.

As a general rule, transparent objects, like Amœbæ and the Infusoria generally, *should be mounted in an aqueous medium for photography*, as this gives better photographic contrast than does a medium having a higher index of refraction, such as glycerine.

If the refractive index of a transparent object is exactly that of the medium in which it is suspended, and it is entirely without color, it is evident that its presence cannot be detected either by the eye or upon a sensitized plate.

The Infusoria are found in great numbers and variety in fresh and salt water, and especially in that which is stagnant and contains considerable organic matter.

Euglena viridis. (Plate III. Fig. 2.)

The species shown in the figure is a common and widely distributed form. The variety of shapes assumed by the creature in its snail-like movement upon the surface of any object to which it attaches itself is well shown; but the red eye-speck and the green color of the endoplasm are of course lost in a photograph.

Spores of Fungi.

The spores of many of the Fungi are suitable microscopic objects to photograph, and the photographic method could not fail to be of value to one especially interested in the study of the fungi.

The deep brown color of some of these spores, however, causes them to arrest the actinic rays so completely that the photograph does not show plainly the internal septa which are characteristic features of certain species (*Coniomycetes*).

The spherical or oval spores of moulds and mildews (*Penicillium*, *Aspergillus*, *Botrytis*, etc.) are better adapted for photography than are the more

deeply colored septate spores referred to. They may be dusted upon the surface of a slide and photographed, dry, without the use of a cover-glass, or they may be mounted in an aqueous medium, or in glycerine, in a very shallow cell. The latter method gives the best results.

To get rid of air-bubbles, which will give great trouble if the attempt is made to introduce the spores at once into water or glycerine, it is best first to wet them thoroughly with alcohol, and before this has entirely evaporated, to place them in the medium which has been selected.

A good plan is to place a drop of alcohol in the centre of a glass slide, and to bring in contact with it a patch of mould in full fruit. The spores will be detached upon contact with the alcohol, and will sink to the bottom of the drop. By a little agitation of the slide they will be distributed in a tolerably uniform layer upon the surface of the glass. When they are nearly dry, in consequence of the evaporation of the alcohol, this is replaced by a drop of distilled water, or of glycerine, and the thin glass cover is applied. The superfluous fluid is removed with blotting-paper (Swedish filtering-paper is the best), and a circle of zinc cement may be turned around the edge of the glass to prevent evaporation while the exposure is being made, or if the intention is to preserve the preparation. A circle of cement is not used to support the margin of the glass cover, as the aim should be to have as thin a stratum of fluid as possible, in order to prevent the spores

from floating about. It may be that mounting in glycerine jelly would be a good plan for the spores having some color, and this method would have the advantage of retaining them in position.

Epithelial Cells. (Animal.)

Epithelial cells are to be obtained from the surface of mucous membranes by gently scraping them with a dull instrument, such as the blade of a pocket-knife, or by applying a thin glass cover, held with forceps, directly to the moist surface of the membrane.

The flat cell with irregular outline, seen in Fig. 1, Plate IV., is from the mucous membrane of the human mouth. Detached cells of this kind will be found in great numbers in the saliva. A little of this fluid spread upon a thin glass cover and allowed to dry will furnish satisfactory fields for photography. Staining agents are required, to show the nucleus. In the example given, the agent used was methyl violet, — ordinary violet ink. The small, deeply colored spherical and rod-shaped bodies scattered over the surface of the cell and in the field around it are Bacteria.

The ciliated epithelial cell, from the mouth of a frog, seen in Fig. 2, Plate III., was not stained, and indeed was still living, as shown by the active movement of the cilia, when the photograph was made. The small spherical body in contact with this cell is a mucus-corpuscle. This photo-micrograph gives a good idea of the spheri-

cal form and transparent contents — protoplasm — of a cell of this kind, and is consequently more satisfactory, as interpreting nature, than the deeply stained cell in Fig. 1. Those, however, who are accustomed to seeing natural objects represented by wood-cuts and with printer's ink, will perhaps prefer the sharper contrast obtained by the use of staining agents.

The scales of the Lepidoptera — butterflies and moths — are suitable objects for photography, and some of them are especially interesting to microscopists for the reason that they are used as tests of the performance of objectives. They may be mounted dry, and extemporaneous preparations are quickly made by applying the wing or body of a lepidopterous insect to the surface of a clean glass slide.

Blood-Corpuscles.

The blood-corpuscles of man and the lower animals are among the objects most suitable for photography. Comparatively high powers will be required; and, for purposes of comparison as to dimensions, it is well to adopt a standard of amplification, say one thousand diameters.

The writer's best results have been obtained with the one-twelfth and one-eighteenth inch homogeneous immersion objectives of Zeiss.

The corpuscles are spread upon a thin glass cover in as uniform a layer as possible, and are allowed to dry *in situ*. They do not require staining, and are mounted, dry, over a circle of cement.

The simplest method of spreading them is to place a small drop of blood on one edge of a glass cover, resting upon a smooth surface, and to draw the end of a glass slide, held obliquely, across the face of the cover. No pressure must be used, or the delicate corpuscles will be crushed and distorted.

In selecting a field for photography, the aim should be to obtain one in which the circular form of the red corpuscles is preserved, in which they do not overlie each other, and in which one or more white corpuscles are to be seen. Unfortunately, an ideal field is hard to find, and the patience of the operator will often be sorely tried in the effort to find one.

The white corpuscles being larger than the red, and spherical in form, are very commonly drawn to the edge of the stain in the operation of spreading. Care must be taken that the blood-stain is quite dry and the circle of cement upon which the cover is to be mounted quite hard, before it is placed in position on the slide; for moisture, or chloroform from the cement, would injure the preparation.

A series of photo-micrographs of blood-corpuscles, made with a standard amplification, would not only be interesting and instructive, but might also be useful for reference, to those who are called upon to examine blood-stains for the purpose of giving expert medico-legal testimony.

The photographic method would also be useful for recording differences in the form and appear-

ance of blood-corpuscles due to disease, if any constant peculiarities of this kind were associated with particular diseases. But the microscope does not reveal any such peculiarities of a sufficiently definite character to justify the expectation, at one time extensively entertained, that its use, in the examination of the vital fluid, might prove of value in deciding questions of diagnosis.

Differences in the relative proportion of the white and red corpuscles are, however, shown in a rough way, and the depth of color of the red corpuscles is indicated, to a certain extent, by the photographic contrast with the ground; or, better still, with white corpuscles in the same field.

The presence of foreign elements — parasitic organisms — is shown very satisfactorily in photo-micrographs; and if a sufficient power is used, their absence is rendered apparent when there are none.

The method is therefore especially useful for recording facts of this kind, as the observer is able to substantiate the truth of his statements, positive or negative, by unimpeachable evidence, and at the same time to show that his skill as a microscopist is sufficient to give confidence in his ability to manipulate the high powers with which such observations are necessarily made.

For example, the photo-micrograph of yellow-fever blood in the present volume, Plate VI., in which the amplification is nearly fifteen hundred diameters, and in which the white and red blood-corpuscles are well defined, may be taken as

evidence that there were no parasites in the blood of the patient from whom this specimen was obtained; and a sufficient number of similar photo-micrographs of blood from different patients, and drawn at different stages of the disease in question would prove the absence of any foreign elements, demonstrable with the power used, from the blood of yellow fever. This has been demonstrated by the writer in the manner indicated for the disease in question. For an example of the use of the method in those cases in which parasitic organisms are present, the reader is referred to Fig. 2, Plate I.

Pollen-Grains.

The diversity of form exhibited by pollen-grains, and the curious markings, bands, and projections upon the external surface of the cellular envelope of the pollen of certain plants, cause them to be interesting objects for the microscope.

Not all of those, however, which it would be most desirable to photograph are suitable objects to be photographed by transmitted light, for the reason that the bright yellow color and comparatively large size of some render them practically opaque. Doubtless this difficulty in the case of pollen-grains, the deeply colored spores of fungi, etc., can be overcome by special methods of preparation, — the use of decolorizing agents, mounting in media of high refractive index, etc. The limits of the present volume do not, however, permit the writer to go very extensively

into details with reference to the preparation of objects, even if the technique were completely worked out, which is far from being the case.

The general statement may be made, however, that *objects which, in water, are not sufficiently transparent for photography, should be mounted in media having a higher refractive index*, of which the most useful are glycerine and Canada balsam.

Much valuable information with reference to the mounting of objects in these media can be obtained from the various works on the microscope, and especially from the files of microscopical periodicals. The best of these is undoubtedly the "Journal of the Royal Microscopical Society" (London), which is published bi-monthly.

Some pollen-grains swell up and the membranous envelope is ruptured when they are immersed in water. For this reason, as well as for that already given, glycerine is commonly a more suitable fluid in which to mount them. When first placed in glycerine, the cell wall becomes collapsed from exosmosis of the watery contents; but after a time the natural form is recovered by endosmosis, and the fluid within and without is of the same density.

To prevent the trouble arising from the presence of air-bubbles, which are apt to adhere tenaciously to the pollen-grains, it is best to immerse them first in alcohol, as recommended for the spores of fungi.

A drop of alcohol is placed in the centre of

a glass slide, and the ripe anthers, held in slender forceps, are brought into contact with it; the pollen is detached, and falls to the bottom of the drop. A little agitation of the slide causes it to be distributed in a stratum consisting of a single layer of cells. When the alcohol has nearly evaporated, a drop of glycerine is put in its place, the thin cover is applied, and the superfluous fluid removed with bibulous paper.

Epidermis of Plants.

The epidermis may readily be stripped from the leaves of many plants, and is well suited for photography. Succulent leaves, especially, like those of the house-leek, ice-plant, various species of lily, century-plant, etc., part with their epidermis easily, while thin and leathery leaves do not.

The well-defined walls, of cellulose, which constitute the outer envelope of vegetable cells give good photographic contrast with the cell contents, and no staining agents are required for its differentiation, as is the case with the cell wall of many cells making up animal tissues.

In the epidermis of plants these vegetable cells are arranged in a single plane to constitute a membrane, and a variety of mosaic patterns are produced as the result of the various forms and modes of arrangement of the individual cells.

In mounting portions of the epidermis for photography, the principal difficulty to contend with is the presence of air-bubbles, which adhere

very tenaciously to the surface, or in the stomata. These may usually be gotten rid of by soaking for a considerable time in water or in alcohol.

When the specimen is thin and quite transparent, water will be the best medium in which to mount it; otherwise glycerine will be preferable.

Plant Hairs.

The appendages to the epidermis of leaves and stems, known as plant hairs, are interesting objects both from a physiological and a morphological point of view. They offer the greatest diversity as to form, and some are very beautiful objects under the microscope. The stellate hairs upon the leaves of *Deutzia scabra* (Plate IX., Fig. 2) are especially well known to amateur microscopists. Stellate hairs are also found upon the leaves of the tree mallow (*Lavatera ascergentifolia*), and doubtless upon many other plants. In the throat of the corolla of snap-dragon will be found a little tuft of very curious and pretty hairs, each of which has a spherical head supported by a slender stalk. Cotton is an elongated vegetable hair.

Some ingenuity will have to be exercised in preparing objects of this kind for photography. Hairs that are closely applied to the surface of the leaf may be photographed *in situ* by mounting the epidermis, or by reflected light. Others will require to be detached, and may be shaved off, with a razor and mounted in a very shallow cell in water or in glycerine.

It is always desirable to obtain a field in which the objects do not overlie or cross each other; and with long plant hairs, like cotton, this is not an easy matter unless they are carefully arranged one by one. A good plan both for long plant hairs and animal hairs is to place several side by side on a dry glass slide, fixing the ends to the edges of the slide with sealing-wax. When they are adjusted in position the central portion is wet with alcohol, then with water, and finally with glycerine, if it is to be used. A thin glass cover is then applied.

Animal Hairs.

A series of photo-micrographs of animal and vegetable hairs would be extremely interesting and instructive. Reagents will often be required to show the structure of animal hairs, which is not so simple as that of those from the vegetable kingdom. The thickness of these hairs makes it desirable that photo-micrographs should be made with low-power objectives, as these have the greatest penetrating power. At the same time good definition is required to show the outlines of the imbricated cells in wool, for example. Wool, ready dyed, of any shade required, is to be had by picking out a little end of colored worsted.

Hints in regard to mounting have already been given in speaking of vegetable hairs, and the limits of this volume permit of little more than hints with reference to this part of the technique,

which, moreover, is as yet only imperfectly worked out. The student, therefore, keeping in mind the general rules which have been given, will have ample scope for the exercise of his own ingenuity.

Sections of Vegetable Tissues.

Photo-micrographs are especially well adapted as illustrations of vegetable histology; and the ease with which sections of vegetable tissues are made and mounted for the purpose, as well as the beauty of the result, cannot fail to make this one of the most popular applications of the art.

Sections — transverse, oblique, or longitudinal — are quickly made with a sharp razor from the petioles of leaves; from succulent stems, like the new growth of asparagus, geranium, etc.; from bulbous roots and tubers; from endogenous plants, such as canna, maize, etc.; and from recent sprouts on exogenous trees and shrubs.

No section-cutter is required for this purpose; and every one engaged in work of this kind should make himself an expert in free-hand section cutting, as many of the best photographs are made from extemporaneous preparations.

The proportion of mounted preparations in animal and vegetable histology, to be found in every collection which are *not* suited for photographing, will surprise one who attempts to save himself the trouble of mounting his own specimens for the purpose.

The first requisite is a very *thin* section; the second, a very *clean* specimen, free from dirt or air-bubbles. To secure cleanliness, wash the leaf or stem or tuber perfectly clean before commencing to make sections, and place the sections in filtered water when they are made. Use a very sharp instrument, and cover the face of the stem, or whatever it may be, with water or alcohol; the razor also should be wet before making each cut.

Be extravagant in the number of sections cut, and select only the best. The selected sections will often require soaking for a considerable time in alcohol, to get rid of the air-bubbles. They are to be mounted in water, solution of acetate of potash, glycerine, or Canada balsam. A little experience will enable the operator to judge whether a section, examined under the microscope in water, requires a medium of higher refractive index in order to render it more transparent. Cells in which the cellulose envelope is comparatively thin, as in the pith of exogenous stems, the epidermis of thin-leaved plants, etc., will show better in water. Thin longitudinal shavings of the wood of the coniferæ — pine, cedar, etc. — may be mounted in glycerine, after being soaked in alcohol to remove air from the cells. Of course water and glycerine may be mixed in any proportion which seems desirable, to secure a refractive index between the two; and it may be that the addition of chloride of cadmium, or chloral hydrate, to glycerine, for the

purpose of obtaining a fluid of still higher refractive index, would in certain cases give still better results.

With a homogeneous immersion achromatic condenser, a homogeneous immersion objective, and a mounting medium having the refractive index of the glass slide and cover, it would seem as if the optical conditions should be as near perfect as possible.

There is ample room for experiment and improvement of the technique, and the writer can commend this fascinating art to those who have patience and are fond of overcoming difficulties, as one well worthy of occupying their leisure time. Moreover, the knowledge of histology gained in searching for suitable objects to photograph will be of a practical kind, like the knowledge of anatomy gained in the dissecting-room, and the time expended in this way will not be lost.

The student of vegetable histology is advised to make sections showing the bark, wood, and pith of exogenous stems (see Fig. 1, Plate XI.); the fibro-vascular bundles in endogenous stems (see Fig. 2, Plate XI., and Fig. 1, Plate X.); spiral vessels, annular and pitted ducts, scalariform ducts (in ferns), etc. He is also advised to make photo-micrographs of the various kinds of starch *in situ* in the cells of succulent shoots, roots, and tubers, — to be mounted in glycerine, — and of raphides (crystals found in the cells of certain plants). Cross sections of the stem of "Wandering Jew" (Tradescantia) will show very beautifully small

spherical starch-grains, and acicular crystals (raphides), enclosed in the large cells of the pith.

The beginner cannot do better than to take a common potato to practise on for his first lesson in cutting thin sections. An extremely thin section, mounted in glycerine, is a beautiful object, but one rather difficult to photograph.

Diatoms.

The silicious frustules of diatoms will always be favorite objects with microscopists, on account of the variety and beauty which they present as to form and markings, and because they serve as tests of the resolving power of objectives. They are especially well adapted for photo-micrography, and the beginner cannot do better than to select for his first attempt one of the least difficult test-diatoms with moderately high powers. *Triceratium favus* (Plate XIII.), which is No. 1 of Möller's "Probe-Platte," is a good one to commence with.

A good half-inch or four-tenths-inch objective will be better than a higher power, as the slightly convex form of the frustule calls for penetrating power. The amplification may be from two hundred to five hundred diameters.

Navicula lyra may be tried next, with a good one-fifth or one-sixth-inch objective, and an amplification of six hundred to one thousand diameters. From this the student may go to *Pleurosigma angulatum*, or *P. quadratum*, using an immersion one-tenth.

These diatoms should be resolved with ease by central light. For the more difficult test-diatoms, and especially for *Amphipleura pellucida*, oblique illumination will be required.

When a considerable number of diatoms are arranged upon a single slide, it is impossible to make satisfactory photographs of all at one exposure, as the focal adjustment and time of exposure which would be best for one is not the best for others. In this case, as already stated in Section IX., the aim should be to get the best average result.

The photographic method is well adapted for the illustration of a work upon the *Diatomaceæ;* but as a matter of economy it would be necessary to arrange several species upon a single plate. The best results would be obtained by making a separate negative for each diatom. These might be made with an amplification considerably above that admissible in the published work, and afterwards reduced to the proper size. For this purpose silver-prints of uniform tone should be made, and the diatoms should be cut out and pasted on a large sheet of white cardboard. A reduced negative should then be made from this, from which the gelatine plate used in heliotype printing could be prepared.

Another method would be for an expert to mount selected diatoms for each plate with special reference to uniformity as to amplification and exposure required.

When a number of negatives are used to make

up a single plate, as in the present volume, these should have as nearly as possible the same tone printing quality; and they will require to be skilfully cut for the purpose.

Diatoms should be mounted in balsam for photography, and the amateur will do well to obtain a slide of arranged diatoms by a skilled preparator. The slides made by J. D. Möller, of Wedel, in Holstein, are celebrated for their beauty and perfection. Plate XII., in the present volume, is from the fourth square of the *Typen-Platte* of this *Präparator*.

Insects.

Small insects, which are not too deeply colored, mounted in balsam, are very good objects to photograph with low powers. The wings, tracheal tubes, feet, antennæ, etc., may also be photographed with higher powers. The proboscis of the blow-fly is a favorite subject.

The suggestion has already been made that photo-micrographs of the larger insects might be made by reflected light with a lens of comparatively long focus but good defining power, and that the enlargement might be made from the first negative, in which the image should be even less than the natural size of the object.

Illustrations of Animal Histology and Pathology.

The beautiful photo-micrographs of histological and pathological preparations by Dr. Woodward, made at the Army Medical Museum, show what

may be done in this direction. But there are obstacles to be encountered which make it necessary that one who hopes to equal these productions of a skilled microscopist shall at least possess a tithe of his patience and persistence in surmounting technical difficulties. The writer has given but little time to this kind of work, having devoted his attention mostly to photographing unicellular organisms, and especially to the Bacteria.

Manuals of histology and of microscopical technology must be consulted by one who proposes to prepare his own specimens for photography, as is strongly recommended.

The following named works will give all necessary information, so far as the technique has been perfected; but the student should not blindly follow any teacher. Keeping in mind the general requirements due to the restrictions imposed by the laws of optics and the optical appliances used, and making himself familiar with the structural details which it should be his aim to show plainly in his photo-micrographs, the operator should avail himself of all the information to be derived from text-books, and at the same time make every effort to improve the technique for this special purpose.

A little work by Professor Schäfer, of University College, London ("Histology and the Microscope"), will be found especially useful by beginners. More advanced students should have in their libraries the following works: "The Microscope and Microscopical Technology," Frey (William Wood & Co., New York); "A Manual

of Histology," Stricker (same publishers); "A Manual of Histology," Satterthwaite, 1882 (same publishers); "The Microscope in Medicine," Beale (J. & H. Churchill, London); "The Micrographic Dictionary (Van Voorst, London).

No microscopist can afford to do without "The Microscope and its Revelations," Carpenter (Blakiston, Philadelphia).

PART II.

DESCRIPTION OF PLATES.

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THE plates in this little work have been selected and arranged not only for the purpose of showing what photo-micrographs are, how they are made, and what microscopic objects are best suited for photography, but also with a view to conveying, by the pictorial method, some elementary biological information.

The writer hopes that the volume will be useful not only to those who propose to make photo-micrographs, but to those who are seeking information, and to whom the wonders of the microscopic world are comparatively known.

The effort will, therefore, be made to give a popular character to this part of the work, and it may be that the title, ELEMENTARY LESSONS IN BIOLOGY, would not be considered out of place at the head of this section. The less pretentious title is, however, selected, with the understanding that if the writer's opportunities permit, and a reasonable demand on the part of the public

justifies, the enlargement of the work, it may eventually be given the title indicated.

The general truth which these photo-micrographs are intended to illustrate is that *the lowest living beings are* UNICELLULAR ORGANISMS, *and that the tissues of higher plants and animals are made up of* CELLS.

The essential part of a *living* cell is a little mass of transparent jelly-like material called *protoplasm.* This is a complex nitrogenous substance, which varies within certain limits as to its consistence, the presence or absence of granules, *nuclei*, *nucleoli*, etc.; but it is essentially the same both in animal and vegetable cells, so far as its chemical composition and reactions are concerned. Its vital potentiality, however, varies within wide limits. This is illustrated by the fact that one little mass of protoplasm enclosed in an envelope of cellulose may be restricted as to its power of development to the simple *rôle* of producing other cells like the first, unicellular organisms; while another similar mass, enclosed in a membrane of different composition, — as, for example, the fertilized ovum of a mammal, — may develop into a vertebrate animal.

Vegetable cells and most animal cells have a *cell-wall*, or limiting membrane. In vegetable cells, this is composed of cellulose, and is well defined. In animal cells, it consists of nitrogenous material, and is often extremely thin, and difficult to demonstrate.

Amœba. (Plate II., Fig. 1.)

The Amœbæ are frequently spoken of as little masses of living protoplasm without organization. This is not quite true, for in Amœba the external surface of the protoplasmic mass has a little more consistence than the interior, and serves the purpose of a limiting membrane; while in the interior may be seen a flattened sphere of granular material, — the *nucleus*, — and a cavity — the *contractile vesicle* — which contracts and expands in a rhythmical manner, and contains a watery fluid. The denser external layer of protoplasm, however, differs from a membranous envelope, inasmuch as it may be perforated at any point without injury, as the fissure is immediately closed by the coalescence of its margins. This is seen in the taking of food and rejecting of effete material from the body of the animal. Food particles are admitted through any part of the exterior of the body with which they chance to come in contact, there being no mouth and no digestive cavity; and undigested remnants are rejected in the same way.

That the nucleus and contractile vesicle are not organs necessary to the creature in sustaining its independent existence, is shown by the fact that a little mass of the protoplasm detached by accident becomes at once an independent amœba, fully able to shift for itself, in which subsequently a nucleus and a contractile vesicle may appear.

The amœba not only eats without a mouth and

PLATE II.

FIG. 1. — AMŒBA from the gutters of New Orleans (May, 1880), magnified 600 diameters ; Beck's one-fifth-inch objective ; heliostat.

FIG. 2. — MICROCOCCI in blood of rabbit (inoculation experiment, Baltimore, 1881), magnified 1000 diameters by Zeiss's one-eighteenth-inch homogeneous immersion objective ; iodine staining ; heliostat.

FIG. 3. — MICROCOCCUS UREÆ (Pasteur), from urine undergoing alkaline fermentation ; magnified 1000 diameters by Zeiss's one-eighteenth-inch objective ; heliostat.

FIG. 4. — AMŒBA PROTEUS, with pseudopodia extended ; magnified 200 diameters by Tolles's four-tenths-inch objective ; heliostat.

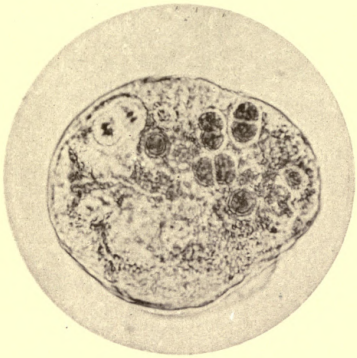


FIG. 1.

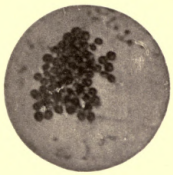


FIG. 3.

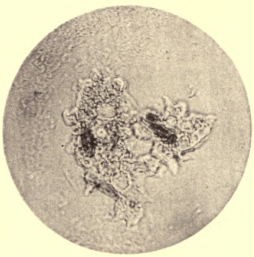


FIG. 4.

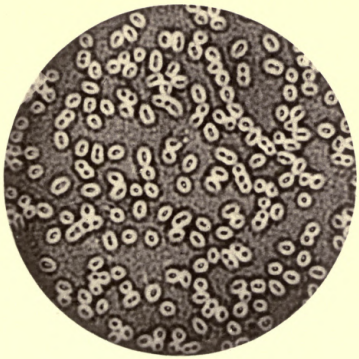


FIG. 2.

digests without a stomach, but it walks, or at least moves from place to place, without feet. This it does by means of what are called *pseudopodia* (pseudopods[1]), or false feet. These are simply projections pushed out from the body-mass; and movement is effected by a flowing over of the fluid protoplasm into the extended pseudopod, not by the attachment of these false feet to the surface along which movement takes place, and by a dragging along of the mass.

The Amœbæ are vegetable feeders, — probably not so much from choice, as because they have no means of capturing the active Infusoria which are associated with them in the stagnant water where they are commonly found. Professor Leidy has seen a living infusorium within the contractile vesicle of *Amœba proteus*.

The Amœbæ evidently have the power of selecting food; for when they come in contact with a unicellular alga or other water-plant of suitable size to be included within the protoplasmic mass, they admit it to the interior by extending pseudopodia around it. These coalesce at the extremities; and in this way filaments several times as long as the diameter of the body of the creature in repose, may be engulphed and digested. On the other hand, inorganic particles with which they come in contact receive no attention, or are only accidentally included when making a meal, and are quickly rejected. As already stated, a detached pseudopod becomes at once an inde-

[1] Leidy.

pendent amœba. This is a common mode of multiplication. Multiplication also occurs by regular binary division.[1]

The Amœbæ are not at all times engaged in the active work of seeking and digesting food. They often remain in a state of repose for a considerable period. This is especially the case during the winter months, when they are to be found in the superficial stratum of ooze in ponds and ditches, and attached to decayed leaves and the roots of aquatic plants. In assuming this encysted condition, they first cease to feed, and get rid of all undigested remnants of food. They assume a spherical form, and the external portion of the body-mass becomes hardened, so as to form a structureless membranous envelope. When conditions are favorable for their renewed activity, the envelope is ruptured, and the semi-fluid, living jelly (protoplasm) flows forth to resume its active and vagabond life.

Professor Leidy thinks it probable that the protoplasm may also be broken up into reproductive "germs or spores" during the encysted state; and one observer, Professor A. M. Edwards, has observed the issue of "swarm-spores," which swim about for a time like Infusoria.

Fig. 1 (Plate II.) is a portrait from life of an amœba quietly engaged in the important occupation of digesting his dinner. This consists of minute unicellular plants (*Protococcus*). The granular material seen is mainly the partially digested

[1] Carpenter.

remnants of former meals. The protoplasm itself presents, however, a slightly granular appearance under high powers.

The Amœbæ belong to a class of microscopic animals which includes a vast number of species, known as *Rhizopods*, or root-footed animals (*rhiza*, root; *pous*, foot).

The Rhizopods are aquatic in their habits, and a large number of the species are found in salt water.

For fuller information in regard to these curious and interesting creatures, the reader is referred to Dr. Carpenter's comprehensive work, "The Microscope and its Revelations," and to the magnificent memoir of Dr. Leidy, "Fresh-water Rhizopods of North America," which constitutes Vol. XII. of Professor Hayden's Report, United States Geological Survey of the Territories. An excellent synopsis of this work has been compiled by Romyn Hitchcock, F.R.M.S.[1] Those who are unable to purchase the work of Professor Leidy will do well to consult it in the nearest scientific library, for the purpose of examining the beautiful plates by which it is illustrated. An hour spent in this way will give a better idea of the variety of forms and general appearance of these lowly creatures than can be obtained in any other way, except by seeking for them in their favorite haunts, as Professor Leidy has done, and by examining the living specimens under the microscope.

[1] Editor and publisher of the "American Monthly Microscopical Journal," 51 and 53 Maiden Lane, New York.

Micrococci. (Plate II. Fig. 2.)

Micrococci are unicellular vegetable organisms, which, as regards dimensions, simplicity of structure, and mode of reproduction, are at the very foot of the scale of living beings. They belong to the class of unicellular plants known as Bacteria, — a class which includes many rod-like forms, and of which all the species are microscopic. These, being larger than the Micrococci, which are little spheres, first attracted the attention of microscopists, and gave name to the class (also called *Schizomycetes*).

The structure of Micrococci is that of vegetable cells generally, viz. a cell-wall, and contained protoplasm. They are so minute that the cell-wall cannot easily be demonstrated under the microscope ; but that it exists, and that it is composed of cellulose, as in other vegetable cells, is shown by the resistance offered to various chemical reagents, — strong acids and alkalies, — which destroy naked protoplasm or animal membranes.

The mode of multiplication is the simplest known or conceivable. A spherical micrococcus, placed in a suitable nutritive medium, becomes elongated, and divides in the median line into two micrococci. Each of these again divides into two, in the same way, by *spontaneous fission*, as it is called, and so on *ad infinitum ;* that is to say, so long as the supply of nutriment holds out, and the conditions as to temperature, etc., are favorable to the growth of the microscopic plant.

Sometimes the cells resulting from this continued division remain attached to each other, and form little chaplets, like strings of pearls. Again, complete division occurs, and the micrococci remain separate, or are grouped together in little masses (*zoöglœa*).

Sometimes division by fission does not occur in one direction only, but first in one direction, and then in a plane at right angles to this. The result is that the micrococci are arranged in groups of four, and not, as in the other case, in a right line, in chaplets.

As multiplication occurs with great rapidity when the conditions are favorable as to nutriment and temperature, it is evident that a small number of micrococci may produce an enormous progeny within a short time. Thus, in the case of the micrococcus seen in Fig. 2, Plate II., the multiplication is so rapid that a single drop of fluid containing the organism, introduced beneath the skin of a large and healthy rabbit, causes the blood to be invaded throughout within forty-eight hours by such a multitude, that the smallest smear of blood-serum spread upon a thin glass cover, and placed under the microscope, gives us such a view as we have in the photo-micrograph.

Cohn, a distinguished German botanist who has given special attention to the study of the Bacteria, makes the following estimate : —

"Let us suppose that a bacterium divides into two in the space of an hour, then into four at the end of the second hour, then into eight at the

end of three hours, in twenty-four hours the number will amount to more than sixteen millions and a half. At the end of two days this bacterium will have multiplied to the incredible number of 281,500,000,000; at the end of three days it will have furnished forty-seven trillions; at the end of a week, a number which can only be represented by fifty-one figures."

The smallest micrococci are less than one-fifty-thousandth (0.5μ) of an inch in diameter. The periods upon this page measure about one-fiftieth of an inch in diameter. A sphere of the size of one of these periods magnified one thousand times would be equal to a spherical mass twenty inches in diameter, — the ball of a twenty-inch cannon, for example; but the micrococcus magnified one thousand diameters would only equal the small sphere represented by the period. Comparing the mass, we find that it is as 1 to 523,600,000.

It is not surprising that these minute plants for a long time eluded the observation of microscopists, and that it is only during the last few years that they have received the study which they deserve. For aside from their interest to biologists as the lowest of living organisms, they have an interest for physicians and sanitarians as the probable cause of certain infectious and epidemic diseases. This is not, however, the place to discuss this question, which is still under investigation.

The aureole of transparent substance around the micrococci in Fig. 2 seems to be peculiar to this

species *when it is cultivated in blood-serum;* at least it is not so marked in other media or in other species.

A careful inspection of the figure will show all the stages in the process of multiplication by binary fission. Some of the micrococci are seen to be quite spherical; others are slightly oval; others long-oval, and among these some are seen to present evidence of commencing division; others, again, show the process of division more or less advanced, or quite completed.

There are different species of Micrococci, which vary as to size, color, and physiological characters. Cohn describes several species which are chiefly distinguished by the presence of different colored pigments, and which he calls *Chromogenes.* One of these is of a "golden-yellow" color, one a "pink-carmine," another a "deep-blue," etc.

In size, they vary from one-half a micro-millimetre (one-fifty-thousandth of an inch) or less, to two micro-millimetres or more.

As among plants higher in the scale, *the size and rapidity of multiplication varies, within certain limits, with the richness of the nutritive medium in which they are placed, and the temperature at which it is maintained. A suitable nutritive medium for one species, however, is not always suited to the development of another,* and considerable diversity exists as regards the most favorable temperature.

A nutritive medium which is exhausted for one species may serve for the development of another.

By the use of the highest powers and of staining

reagents, it will be seen that *considerable differences as to size occur in different individuals of the same species* and in the same culture medium.

These facts, which have all been verified by the personal observations and experiments of the writer, make it evident that the laws of natural selection have full play among these lowly microscopic plants, as well as among those higher in the scale.

Micrococci are widely distributed in nature; they are constantly floating in the atmosphere in a desiccated condition, or in the shape of ultra-microscopic germs; and when they are deposited in a liquid or upon a moist surface, where the conditions are favorable for their growth, they quickly resume their reproductive activity.

If a drop of fluid containing micrococci — as, for example, a drop of blood from a rabbit, just dead, containing the species shown in the figure — be added to a transparent "culture-fluid" such as *bouillon* made from the flesh of a rabbit, in a "sterilized" flask, and this be maintained at a temperature suitable for the multiplication of the little plant (98° — 100° for the species under consideration), the fluid will become opalescent or even milky in appearance within twenty-four hours, as the result of the rapid increase which has taken place. The presence of these minute organisms, when they are in sufficient abundance, may then be recognized by the naked eye. The colored species are especially likely to develop on cooked alimentary substances. Cooked potato is a good

nutritive base for several of these, and their presence will be recognized by the bright color of the little gelatinous masses or stains produced by their aggregation.

Micrococci abound in the pus from open wounds, and are also found in that drawn from closed abscesses of an acute character. Milk, pus, and perspiration are sometimes colored red or blue, in consequence of the presence of micrococci having these colors.

Micrococci are liable to be mistaken for granular matter of organic origin; but usually the mistake is the other way, and organic or inorganic granules are mistaken for micrococci. The vibratory movement — *Brownian movement* — by which very minute particles suspended in a liquid are seen to be agitated, often leads to mistake by those who are unfamiliar with this molecular movement. Micrococci are also subject to this oscillatory motion, which should not be confounded with vital movements, in which the organism is visibly transported from place to place by virtue of some motive-power residing in its own organization, and independent of any currents in the fluid in which it is immersed. Independent vital movements of such a nature the micrococci do not exhibit; but many of the micro-organisms of the class to which they belong are endowed with movement, and may be seen swimming actively across the field of view, — some simply in right lines; others in a jerky, oscillatory way; others in a serpentine manner; and still others in a spiral direction.

These rod-shaped, filiform, and spiral Bacteria belong to different genera from that under consideration, — *Micrococcus*, — and the limits of the present volume do not permit of any extended account being given of them in this place. They are all more or less elongated, and all multiply by transverse fission in a plane at right-angles to their long diameter. A number of species are also known to multiply by the formation of *endogenous spores*. These are simply little masses of protoplasm formed in the interior of the rods or filaments by the consolidation of a portion of the semi-fluid protoplasm of the parent organism.

These spores are spherical or oval in shape, and when set free by the breaking-up of the cell-wall of the mother-cell, may be mistaken for micrococci. They refract light more strongly, however, on account of their denser structure; and when placed in a suitable "culture-fluid," they grow into rods like the parent form, instead of producing spheres by binary fission, as do the micrococci.

Pathogenic micrococci — that is to say, disease-producing micrococci — have been proved to be the cause of certain infectious diseases among the lower animals, and it seems probable that other species may be the cause of certain infectious and epidemic diseases which afflict the human race. A minute micrococcus is constantly found in the pus of small-pox pustules and in recent vaccine virus. The veritable cause of the infective virulence of the material in which they are found is as yet not definitely settled; and in the

case of other diseases it may be said that much more has been claimed than has been proved. The whole question is still under investigation, and it would be premature to express a positive opinion as to the *rôle* of these organisms in the diseases in question, except as regards those diseases of animals in which it has been definitely established by the experimental method, — fowl-cholera, and septicæmia in rabbits.

Those who desire fuller information concerning the Bacteria, are referred to the writer's translation of Magnin.[1]

Unicellular Algæ. (Plate III. Fig. 1.)

The group from nature seen in Plate III. Fig. 1, is quite a model one for showing the mode of division of a unicellular organism undergoing rapid multiplication by *spontaneous fission*, — binary division.

Every step in the process can be traced by referring to different cells, from commencing transverse division in one direction to complete division with commencing fission — at a right angle with the first line of division — in each cell of the pair.

The Micrococci are, according to Cohn, the distinguished German botanist, unicellular algæ, although Nägeli and others class them with the Fungi. The chief distinction between these two classes of cryptogamic plants consists in the fact that in one (the Algæ) the green coloring matter

[1] The Bacteria. Boston: Little, Brown, & Co. 1880.

known as chlorophyl is present, and not in the other. Moreover, the Algæ are all *water-plants;* and in their growth they appropriate directly inorganic materials held in solution in the medium in which they live. On the other hand, the Fungi are chiefly air-plants; and they derive their sustenance for the most part from other plants, which they infest as parasites, or from organic material supplied by the tissues of plants and animals undergoing decomposition.

The green coloring matter referred to (chlorophyl) cannot commonly be distinguished in the minute algæ known as Bacteria; but in the larger unicellular algæ, the green color is very pronounced. In the autumn, when the approach of cold weather interferes with the active life of these little plants, this green color frequently changes to a ruby red. In certain species, this red color of the protoplasm is a constant character. This is true of *Protococcus nivalis,* which is found upon the snow in the arctic regions and upon snow-covered mountains in lower latitudes, where it sometimes exists in such abundance as to give the snow the appearance of being covered with blood.

The life-history of these unicellular algæ is not so simple as is that of the *Micrococci,* — in which, so far as we know, the only mode of multiplication is by binary division. This mode of reproduction occurs, as seen in the figure. But we have another mode of reproduction among these lowly plants, also observed among unicellular animal organisms, which is very remarkable, and is essen-

tially what occurs in sexual reproduction among plants and animals higher in the scale, viz., the coalescence of two cells to form a new individual.

This process is called *conjugation*, and it is described as follows by Dr. Carpenter, as it occurs in Palmoglœa: —

"The conjugating process commences by the putting forth of protrusions from the boundaries of two adjacent cells, which meet, fuse together, and form a connecting bridge between their cavities. The fusion extends before long through a large part of the contiguous sides of the two cells; and at last becomes so complete that the combined mass shows no trace of its double origin. It soon forms for itself a firm cellulose envelope, which bursts when the '*zygospore*' is wetted; and the contained cell begins life as a *new generation*, speedily multiplying, like the former ones, by binary division."

This evidently corresponds with what occurs in sexual reproduction, which is the only mode of increase among the highest plants and animals. In the unicellular alga, however, the two cells are apparently alike; while in flowering plants and in animals they differ in appearance.

Conjugation in phænogamous plants occurs by the falling of the pollen — the male element — upon the pistil, and the extension of a slender tube from the pollen-grain down to the germ-cell — female element — contained in the ovule. Through this tube the contents of the pollen-grain are discharged, and we thus have a commingling

of the protoplasmic contents of the two cells. The result of this conjugation ("fertilization") is that a seed is formed containing an embryo plant, which, when suitably placed, develops into a new individual like the parent form.

The propagation of higher plants by cuttings may be compared to the mode of multiplication by binary division seen in our figure.

The life-history of some unicellular Algæ also includes a phase in which the little plant, instead of quietly remaining in one place and devoting itself to the business of raising as large a family as possible, assumes a *motile condition* and leads a vagabond life for a time.

This is especially well seen in *Protococcus*, the genus to which the red snow-plant belongs, and one species of which, in the "still" condition, is probably represented in our figure.

Dr. Carpenter says: —

"When the ordinary self-division of the 'still' cell into two segments has been repeated four times, so as to produce sixteen cells, and sometimes at an earlier period, the new cells thus produced assume the 'motile' condition, being liberated before the development of the cellulose envelope, and becoming furnished with two long vibratile *flagella*, which seem to be extensions of the colorless protoplasm-layer that accumulates at their base, so as to form a sort of transparent beak. In this condition it seems obvious that the colorless protoplasm is more developed relatively to the coloring matter than it is in the 'still' cells; and it usually

contains 'vacuoles,' occupied only by a clear aqueous fluid, which are sometimes so numerous as to take in a large part of the cavity of the cell, so that the colored contents seem only like a deposit on its walls.

"The flagella pass through the cellulose envelope, which invests their base with a sort of sheath; and in the portion which is within this sheath no movement is seen. During the active life of the 'motile' cells, the vibration of these flagella is so rapid that it can be recognized only by the currents it produces in the water, through which the cells are quickly propelled; but when the motion becomes slacker, the filaments themselves are readily distinguishable, and they may be made more obvious by the addition of iodine.

"The multiplication of these 'motile' cells may take place in various modes, giving rise to a great variety of appearances. Sometimes they undergo a regular binary subdivision, whereby a pair of motile cells is produced, each resembling its single predecessor in possessing the cellulose investment, the transparent beak, and the vibratile filaments, before the dissolution of the original investment. Sometimes, again, the contents of the primordial cell undergo a segmentation in the first instance into four divisions," etc.

The different appearances presented by these unicellular plants during different stages of their existence led the earlier observers to mistake the different forms for independent species, and the motile forms were very commonly regarded as ani-

malcules. One unfamiliar with the facts recorded would very naturally suppose that these actively moving organisms were little animals, as active movement is a characteristic associated in our minds with animal life. In the microscopic world, however, this is not a distinguishing characteristic; for we not only have plants which move freely from place to place, but we have animals which are permanently located in one position.

It seems probable that the main object of these "motile" forms is to secure the extensive distribution of the species. Among flowering plants this is accomplished in a variety of ways, and especially by means of appendages which aid in the dissemination of the seeds by the winds or by animals.

Without this provision for their preservation, our little plants would be liable to become extinct, both because they would exhaust the nutriment necessary for their growth if restricted to a few localities, and because the animals which feed upon them would have too great an advantage if they always remained at home to be eaten.

FIG. 1,

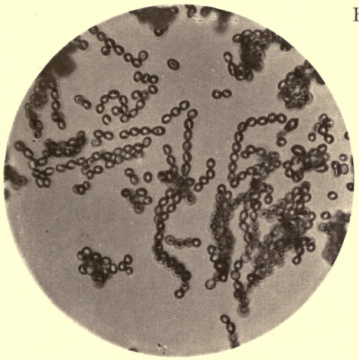


FIG. 3.

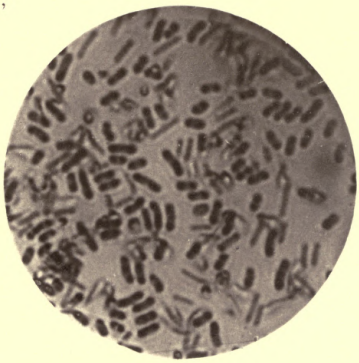


FIG. 4.

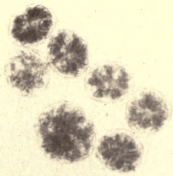


FIG. 2.

PLATE III.

FIG. 1. — UNICELLULAR ALGA from standing water, New Orleans, 1880 ; magnified 400 diameters by Beck's one-fifth-inch objective ; not stained ; heliostat.

FIG. 2. — EUGLENA VIRIDIS from surface of moist mud; magnified 200 diameters by Powell & Leland's one-half-inch objective ; not stained ; heliostat.

FIG. 3. — SPORES OF FUNGUS (*Penicillium glaucum*), New Orleans, 1880 ; magnified 400 diameters ; Beck's one-fifth-inch objective ; mounted dry ; heliostat.

FIG. 4. — BACTERIA which appear to contain endogenous spores. From swamp-mud, culture experiment in gelatine solution, New Orleans, 1880 ; magnified 1000 diameters by Zeiss' one-eighteenth-inch objective.

Euglena viridis. (Plate III. Fig. 2.)

The curious unicellular organism of which we have a photograph in Plate III. Fig. 2, resembles the Algæ in having a bright-green color, — not, however, in the form of granules, but distributed through the "endoplasm," so as to give it a uniform green tint, even when examined with the highest powers. This green coloring matter seems to be similar to, if not identical with, the chlorophyl of plants; and the organism in question is classed with the unicellular Algæ by some naturalists, while others place it with the Infusoria, — the lowest animal organisms.

Dr. Carpenter says: "If, as appears from the recent observations of Bütschli, the well-known Astasia — of which one species has a blood-red color, and sometimes multiplies to such an extent as to tinge with it the water of the ponds it inhabits — has a true mouth for the reception of its food, it must be regarded as an animal, and separated from the Euglena (with which it has been generally associated), — the latter being pretty certainly a plant, belonging to the same group as Volvox."

On the other hand, Kent, the latest and highest authority upon the Infusoria, describes the Euglena as "free-swimming animalcules," and presents evidence to show that they have an oral aperture. He says: "The apparent absence of a distinct oral apparatus and ingestive cavity, taken together with the green hue of the body-plasma, previously

induced most modern authorities, including Mr. Carter, to deny to the representatives of Euglena and its allies the rank of animal organisms, they referring them rather to the division of the Protophyta, or lower unicellular plants. The present author's views, to within a comparatively recent date, harmonized with this decision; but a still later and more exhaustive examination of the type-form of the genus *E. viridis*, made in April, 1877, has resulted in an entirely opposite opinion. Keeping animalcules of this species for a prolonged interval in water, with finely pulverized carmine, and submitting them to a magnification of eight hundred diameters and upwards, the passage into their bodies, at the anterior extremity, of exceedingly fine particles of this pigment was repeatedly observed, as also the accumulation, in various parts of the body-substance, of small globular aggregations of the particles ingested. Hitherto, this anterior region of Euglena has been represented as exhibiting a bilabiate aspect, and the flagellum as a thread-like extension of the upper of the two lip-like prominences. With the aid, however, of the high magnifying power employed on this occasion, it was clearly shown that the inner surfaces of these lip-like prominences actually represented the upper and lower boundary-walls of a conical or infundibular excavation or vestibule, the innermost recess of which fulfils the office of an oral aperture by permitting the free passage of exceedingly minute food-particles."

We have been obliged to concede that it may be endowed with the capacity and the apparatus (flagella) for active locomotion; but a plant with a mouth would be too great a tax upon our imagination, notwithstanding the fact that the creature contains chlorophyl and starchlike granules.

In allowing, however, to Euglena the rank to which its microscopic mouth entitles it, we must not forget that the distinction between plants and animals is not sharply drawn among these lowly organisms, and that the lines drawn by naturalists in their attempts to classify the living forms that come under their observation, are to a great extent arbitrary and artificial. Nature is continuous; but the links in the chain were not all forged at one time, nor is it possible for the most industrious naturalist carefully to study all the forms existing to-day. Consequently, breaks occur in his chain; and these are erected into generic and specific barriers, which are constantly being changed as new discoveries are made.

Our photo-micrograph of *Euglena viridis* shows the form of the creature when at rest, but does not show its green color, its red "eye-speck," or its flagellum, which is not always present.

Extended green patches are frequently seen upon the surface of the moist mud along the margins of the open gutters of New Orleans, which upon examination with the microscope are found to be made up of these little creatures. When at rest, they resemble so closely the unicellular Algæ that it is hard to convince oneself that they

are really animal organisms. But when we see these little green spheres stretch themselves out and crawl about like so many microscopic snails, or, when there is sufficient water for navigation, swim freely about by means of their slender, whip-like flagellum, we can scarcely fail to agree with Kent in assigning them to the animal kingdom.

The red pigment spot — so-called eye-speck — is situated near the anterior extremity of the body. Its function has not been determined, but it is not improbable that it is a rudimentary sense-organ.

Euglena, like Amœba and the unicellular Algæ, may assume an " encysted " condition, and remain quiescent for an indefinite period. This provision of nature for the preservation of these minute beings enables them to survive during the cold of winter and periods of drouth, and to resist desiccation to such an extent that they may be wafted through the air in dust from the bottom of a dried-up pond or gutter; and if they are fortunate enough to fall into an open cistern or any other body of water, they quickly resume their active state.

Euglena multiplies by *fission* in a longitudinal direction. This mode of reproduction is common among the Infusoria; and some much higher in the scale, and unmistakably animal organisms, exhibit this curious phenomenon. The writer had, two years ago, the opportunity of observing the whole process in a very curious creature belonging to the genus Amphileptus. This is a ciliated infusorium having an oval body and a long neck.

It is rather sluggish in its motions, so far as change of locality is concerned; but the long and flexible neck is kept in constant motion in its active search for food, and the cilia upon the surface both of body and neck are constantly in rapid vibration.

An individual of this species being in the field of the microscope, it was noticed that there was a slight constriction in the centre of the body transversely to the long axis of the creature. A microscopical friend happening to call at this moment, it was determined to follow the whole process of division, which was evidently commencing. The creature might be compared, in a rough way, to a glass flask with a long neck; and nature was about to make two such flasks from a single one — very much as a glass-blower would accomplish the same thing — by a constriction in the middle line of the body, a drawing out of the material to form a new neck for the posterior individual, and finally a complete separation of the two at the point of union between the body of the anterior and the end of the neck of the posterior member of the pair.

In the case of the living animalcule, however, the creature itself took an active part in the operation. This was especially the case after the constriction had progressed so far as to leave an isthmus between the two portions of about the diameter of the neck. Vigorous pulling and tugging took place between the bodies, until a neck of proper length had been constructed for the posterior member of the firm; and now the con-

stricting process was resumed at the extremity of this neck, and the efforts of the two perfectly formed individuals were directed to the completion of the divorce. This was not long delayed; and when the last tie was severed, the two worldly creatures, apparently indifferent to the fact that they had been literally one flesh, set out upon independent careers of sensuality,—their whole aim in life seemingly being to secure as large a share of food as they could possibly digest. Fortunately for them, the corpulence which results from high living had its remedy in the simple process already described, by which an overfed and unwieldy body, instead of being reduced by abstinence or "anti-fat," could be remodelled to make two bodies of respectable dimensions. The whole process of division occupied something more than two hours.

To return to our *flagellate* infusorium, *Euglena viridis*. Reproduction occurs not only by binary division, but also by the formation of *endogenous spores* from the protoplasmic body-contents of encysted individuals. Conjugation or coalescence of two individuals sometimes occurs prior to encystment. The globular, spore-like bodies, when released by rupture of the cell-wall of the "mother-cell," appear "as small green creeping amœbæ, possessing at this early stage no trace of the flagellum, oral aperture, or pigment spot" (Kent).

Having made the acquaintance of *Euglena*, the reader will hardly be satisfied until he has extended his knowledge of the Infusoria by direct

observation under the microscope, or by reference to the comprehensive and beautifully illustrated work of Kent, recently published.[1]

Spores of Fungi. (Plate III. Fig. 3.)

The oval bodies, arranged in a linear series, seen in Plate III., Fig. 3, are the spores of the well-known blue mould, *Penicillium*, which is so often found by housekeepers upon the surface of their preserves, upon articles of leather left in a damp place, etc. These spores are the seeds of this microscopic fungus, and they are produced in immense numbers by a process which does not differ materially from that by which the multiplication of the Bacteria is effected — fission. In place, however, of simple binary division, a whole row of spores is formed at the same time by the breaking up of a filament, an off-shoot from the parent plant, into a chain of unicellular elements, — spores. Each of these cells, as in the case of the Bacteria and of other organisms which multiply by binary division, contains a little fragment of the protoplasm of the mother plant enclosed in a membranous envelope.

When one of these spores falls upon a surface where the conditions are favorable to its growth, it quickly germinates, and produces a plant like that from which it was derived. The necessary conditions are a nutritive substratum, a sufficient degree of moisture, and a proper temperature. If

[1] "A Manual of the Infusoria," by W. Saville Kent, London.

upon the surface of a glass slide we spread a little sweetened mucilage, and upon this soil sow the seeds of the microscopic plant by gently applying the surface to a patch of mould in full fruit, we can quickly raise a luxuriant crop, and, under the microscope, can watch the development from hour to hour. For this purpose the glass slide must be kept in a moist atmosphere, and if the temperature be somewhat elevated, 90°–100° Fahr., the growth will be all the more rapid. The whole process is very simple, and consists, in the first place, of the outgrowth from the spore of a slender shoot, the interior of which is filled with protoplasm continuous with that in the spore itself, and being, in fact, an extension of this, resulting from the imbibition of water and of nutrient material.

In some species this filament continues to grow in length and to give off side branches without the formation of any septa in the ramifying *mycelium* which results from this mode of growth. In this case we have evidently a unicellular plant, notwithstanding the linear extension and complicated ramifications which result from the continued growth of the plant.

In other species, transverse septa are developed at intervals, and we have a linear series of elongated cells originating from the little mass of protoplasm enclosed in the first instance in the cell-wall of the oval or spherical spore.

From this branching mycelium, little upright stems or *pedicles* are given off, and these divide at the summit into several slender filaments which

break up into spores like those from which the little plant originated. (Plate III. Fig. 3.)

This is the simple history of non-sexual reproduction: a fragment of protoplasm detached from the parent forms a new individual.

In like manner, in sexual reproduction, little masses of protoplasm, — sperm-cell and germ-cell, — are detached from each parent, and these coalesce to form the fertilized ovum from which a new individual is developed. The spores of which we have spoken are produced in such abundance that there is little chance of the extinction of species from scarcity of seed. This seed is borne far and wide through the air, and whenever conditions are favorable for its germination, there it is sure to find its way, and we have a growth of *Penicillium*, — blue mould. But this, although one of the most common and widely distributed of the microscopic Fungi, is only one out of a multitude of species. Many of these are parasitic upon higher plants, where they constitute the pests known as mould, mildew, rust, smut, rot (potato-rot), etc. The important part which the Bacteria and the microscopic Fungi play in the economy of nature is a recent revelation of science. Formerly, it was supposed that these organisms were present in substances undergoing putrefaction or fermentation, because the products evolved during these processes were suitable for their nourishment. Now it is known that these processes depend upon the presence of the living organisms, and that the most putrescible substances may be preserved in-

definitely if these microscopic plants do not obtain access to them.

The materials drawn from nature's storehouses, — the earth, the ocean, the atmosphere, — for the construction of the higher plants and animals, would be exhausted in course of time if there were no provision for returning them to the common stock; and the surface of the earth would be encumbered with the bodies of successive generations of animals and with dead but undecayed vegetable tissues, were there no provision for the decomposition of these organic structures. This work of decomposition, however, is provided for, and it is accomplished by these microscopic plants, which make up for their diminutive size by their rapid growth and reproductive activity. But, as we have already seen, these little giants do not alone confine their attention to dead organic matter. They also invade the living structures of plants and animals, and appropriate to themselves the material necessary for the vigorous growth of their host, thus causing disease, and sometimes death.

Fortunately, living protoplasm possesses an inherent power of resistance against these parasites, and it is little liable to suffer from their attacks, unless enfeebled by age or want of proper nutriment. The starved plant is more subject to be attacked than the vigorous one. The puny and ill-nourished child is more liable to suffer from parasitic (vegetable) skin diseases than the robust. The individual enfeebled by starvation, wasting

discharges, or any other cause, is especially liable to fall a victim to those epidemic diseases which are supposed to be of parasitic origin.

Many parasitic fungi will only grow upon a particular kind of plant, or even upon a special variety produced by cultivation. Thus, a hardy native rose may go unscathed, while a cultivated variety in its immediate vicinity suffers from the attacks of mildew.

Those who desire fuller information in regard to the microscopic fungi are referred to the Micrographic Dictionary, which gives all the information which amateur microscopists are likely to require.

Epithelium Cells. (Plate IV. Figs. 1 and 2.)

If a little drop of saliva from the mouth be examined under the microscope, it will be found to contain numerous flat cells, of somewhat irregular form, which have been shed from the surface of the mucous membrane lining the cavity. By the use of staining reagents, it will be seen that these cells have a *nucleus* — seen in the centre of the cell in the figure — and a *nucleolus*. The deeply-stained spherical and rod-shaped bodies seen upon the surface of the epithelium cell in Fig. 1 are *Bacteria*.

Epithelium cells of different forms, and somewhat differently arranged, make up the superficial layer of all membranes lining the cavities of animal bodies; and the cuticle or epidermis

which covers the exterior of the body is made up of similar cells.

The epithelium of mucous membranes and of the external surface of the body is constantly being shed. In hospital wards and other places where human beings are crowded together, the dust suspended in the atmosphere or deposited upon floors, window-ledges, etc., is to a considerable extent composed of this shed epithelium, *unless cleanliness is rigidly enforced.*

It would be out of place, in the present work, to go into details as to the differences presented by epithelium from various localities; but attention is called to the very curious form of cell seen in Plate IV. Fig. 2.[1] This is what is known as a ciliated epithelium cell; and the peculiarity consists in the presence upon one side — that which faces outward when the cell is in position upon the surface of a mucous membrane — of a quantity of vibratile filaments called cilia.

This ciliated epithelium lines the air-passages of vertebrate animals; and the office of the vibrating filaments, which are of uniform length, and which move in unison, seems to be to prevent the accumulation of the natural secretions of the mucous membrane by propelling them in the direction of the orifice of the respiratory apparatus.

This ciliary action may continue for some hours after a cell is detached from its normal position; and such a cell, immersed in water of a proper

[1] An accident to the negative has made it necessary to substitute another figure for the one here described.

temperature — blood-heat — may be seen, as the result of this active vibration of its cilia, to swim about like a unicellular organism.

The cell seen in the figure is from the back part of the mouth of a frog. Specimens are readily obtained from this situation by scraping the surface with a dull knife. These ciliated epithelium cells are not shed so freely as the "pavement" or "tessellated" epithelium; but at the outset of a sharp attack of nasal catarrh, they may be found in the watery fluid discharged from the nostrils.

Some years since, some of these ciliated epithelium cells found in the discharge from the nostrils of persons suffering from "summer catarrh," or "hay fever," led to the announcement that the cause of this trouble had been discovered, and that it was due to the presence of parasitic organisms. These organisms were described and figured; but the discoverer has not been heard from since the learned Professor Leidy pointed out the fact that they were nothing else than the ciliated epithelium cells found upon the bronchial and nasal mucous membranes of healthy persons.

Scales of Insects. (Plate IV. Figs. 3, 4, and 5.)

If a little of the dust which adheres to the fingers after handling a moth or a butterfly (Lepidoptera) be placed under the microscope, it will be seen to present the appearance seen in Plate IV. Figs. 3, 4, and 5.

These scales are often very beautiful objects,

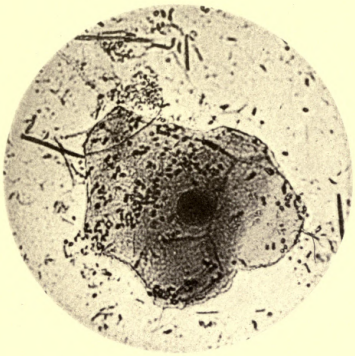


FIG. 1.

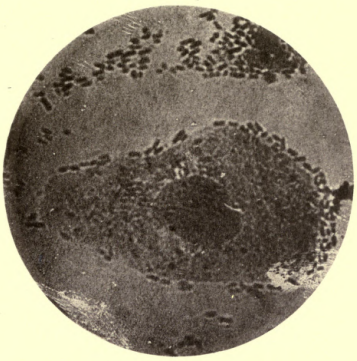


FIG. 2.

FIG. 3.

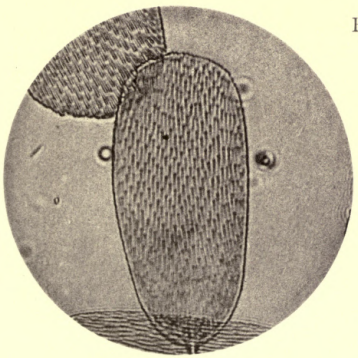


FIG. 4.

FIG. 5.

PLATE IV.

FIG. 1. — EPITHELIUM CELL from human mouth; magnified one thousand diameters by Zeiss's one-twelfth-inch objective; methyl-violet staining; heliostat.

FIG. 2. — EPITHELIUM CELL from extremity of male urethra, surrounded by bacteria commonly found in this location; magnified 1000 diameters by Zeiss's one-eighteenth-inch objective; methyl-violet staining; heliostat.

FIG. 3. — Scales of butterfly (*Satyrus zanira*); magnified 100 diameters by Tolles's four-tenths-inch objective.

FIG. 4. — Podura scale; magnified 1000 diameters by Zeiss's one-eighteenth-inch objective and Tolles's amplifier.

FIG. 5. — SCALES from wing of mosquito magnified 200 diameters by Powell and Lealand's one-half-inch objective.

both because of their varied and curious shapes, and on account of the brilliant colors which some of them exhibit. They may be compared with the epithelium cells covering the surface of animals, and also with the feathers of birds. Like the latter, they not only protect the surface covered by them, but they contain the pigments used so lavishly by nature in the decoration of the insects of this family. The metallic lustre and iridescence exhibited by many of these scales is, however, due to their structure, and not to the presence of pigment.

The scales are arranged upon the wings and bodies of these insects in imbricated rows, in which they overlie each other like the shingles upon the roof of a house. Each scale is held in place by a projection at the inner and acute extremity, which is inserted into a socket provided for its reception upon the surface of the body or wing.

Much attention has been given by microscopists to the study of these scales, with reference to their structure and the nature of the minute markings which are found upon many of them. For information upon this subject, the reader is referred to the work of Dr. Carpenter.[1]

[1] "The Microscope and its Revelations," 6th edition, pp. 723–733.

Blood-Corpuscles. (Plates V. and VI.)

The red color of the blood of animals is due to the presence of a multitude of colored disks, either circular or oval in form, which are suspended, together with other colorless corpuscular elements, in a clear fluid, — the blood-plasma.

The red blood-corpuscles vary as to shape in different classes of vertebrate animals, being circular in all the Mammalia except the camel family, and oval in birds, reptiles, and fishes. They also vary greatly as to dimensions in different genera of the same class. The smallest corpuscles known are those of the musk-deer, which only measure $\frac{1}{12\,\frac{1}{3}25}$ of an inch in diameter; while those of the amphiuma, a reptile common in Louisiana and other Southern States, measure thirty-five times as much, being $\frac{1}{3\,\frac{1}{4}5}$ of an inch in diameter.

Measurements of blood-corpuscles always represent the average obtained by measuring a considerable number; for differences exist in the diameter of corpuscles contained in the same drop of blood.

In man, the red blood-corpuscles measure about $\frac{1}{3\,\frac{1}{2}00}$ of an inch in diameter. The blood-corpuscles of the dog and of the Guinea-pig approach so nearly in size those of man, that it is pretty generally agreed among *expert* microscopists that the attempt to decide positively, by means of microscopical examination and measurements, that certain blood-stains submitted for examination in

medico-legal cases are the blood of man, and not of one of the lower animals named, is likely to result in serious error and injustice to the individual whose life may be imperilled by such unwarrantable testimony.

If the question was as between man and a fowl or reptile, or one of the Mammalia in which there is a considerable difference in size, — as a sheep or goat, — there would be no difficulty in determining that the blood was *not* that of man.

The red blood-corpuscles of birds, fishes, and reptiles have a well-defined central mass or *nucleus*, which is not present in those of *adult* mammals, but is found in the earlier period of the development of the embryo of man and of the lower animals of this class. The oval blood-corpuscles of the Camel tribe have no nucleus.

The general form of the red blood-corpuscles is well shown in our photo-micrographs (Plate V.); but it must not be supposed that this form is rigidly preserved. On the contrary, these little colored, biscuit-shaped, or oval masses are extremely flexible, and either in the capillaries of a living animal or under a thin glass cover may be seen to change their shape from contact the one with another, or from the slightest pressure.

The average number of red blood-corpuscles in a cubic inch of human blood has been estimated at upwards of *eighty millions*. The number is considerably less in certain chronic diseases; and the degree of impoverishment may be estimated by counting under the microscope the number of

corpuscles in a given quantity of the fluid, by means of a hæmacetometer.

After a considerable reduction of the number of red corpuscles as the result of hæmorrhage, the usual proportion is often regained in an astonishingly short period. This fact shows that nature has provided liberally for their continued supply, to make up for deficiencies resulting from sudden loss, or from the disintegration which is no doubt constantly taking place in the circulation.

Much difference of opinion has existed among physiologists as to the mode of origin of these corpuscles; but it is now pretty well settled that a considerable proportion, at least, take origin from the *colorless blood-corpuscles*, about to be described. These colorless corpuscles are found in the blood in considerably less numbers than the red, and the relative proportion fluctuates greatly at different times. Thus, after a prolonged fast, they may be reduced to the proportion of one in a thousand; while after a hearty meal, this proportion may be increased to one in three or four hundred. Their relative proportion is also greatly increased in certain diseased conditions.

One or more white corpuscles are to be seen in each of the figures (Plates V. and VI.); and it will be noticed that in the photo-micrographs of the blood of yellow-fever patients, they contain certain highly refractive granules not seen in the blood from healthy animals (Plate V.). These granules are believed to be fat, either developed in the protoplasm of the corpuscles, or picked up from the

PLATE V.

FIG. 1. — BLOOD OF GUINEA-PIG (Havana, 1879); magnified 1450 diameters by Zeiss's one-eighteenth-inch homogeneous immersion objective, and Tolles's amplifier; spread and dried on glass cover; heliostat.

FIG. 2. — BLOOD OF PIGEON (Havana, 1879); magnified 1450 diameters by Zeiss's one-eighteenth-inch objective, and Tolles's amplifier; spread and dried on glass cover; heliostat.

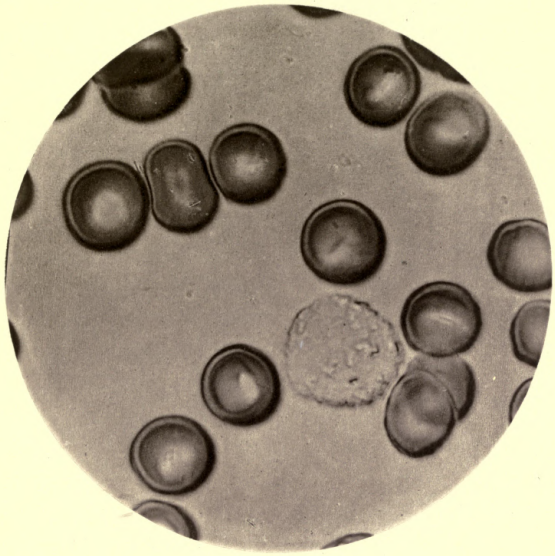


FIG. 1.

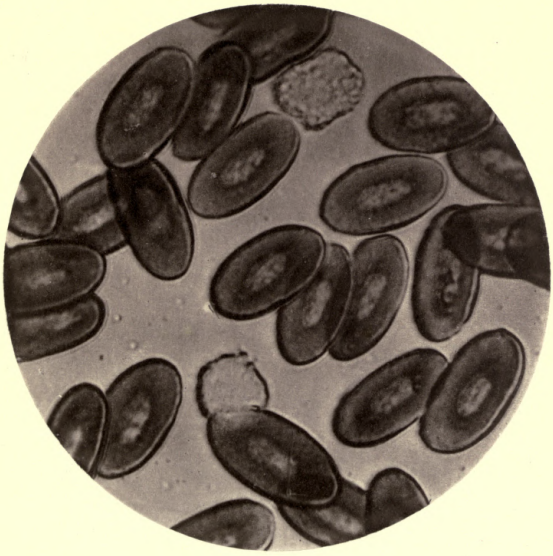


FIG. 2.

blood-serum by them. The latter hypothesis is not at all improbable, for these little masses of protoplasm have been seen to pick up granules which come in their way in the same manner as the amœba takes its food. For, like the Amœbæ, *the white blood-corpuscles are naked masses of living protoplasm, capable of undergoing active amœboid movements, and also of multiplication by spontaneous fission.*

To return to the granules in the white corpuscles in yellow-fever blood, it is also possible that these are developed *in situ*, — as the protoplasm of the cells in the liver and other organs in this disease is changed to a greater or less extent into fat (retrograde metamorphosis), and that of the white corpuscles may perhaps undergo the same change. Be this as it may, the writer has seen these corpuscles carrying along their enclosed fat-granules, and moving around upon the surface of a glass slide — under cover, to prevent evaporation — forty-eight hours after the blood had been removed from the finger of a patient. Not because they were in the blood of a fever-patient, but because the warm climate of Havana is favorable for the long-continued exhibition of this remarkable phenomenon, which may be witnessed at any time by placing a little drop of blood from the finger of a healthy person upon a glass slide supported upon a hot stage, by which the temperature is maintained at about blood-heat.

The white corpuscles may pass from the blood-vessels to the tissues, and when a local inflam-

PLATE VI.

FIG. 1. — BLOOD of yellow-fever patient, Havana, 1879 (first day of sickness, fatal case), magnified 1450 diameters by Zeiss's one-eighteenth-inch homogeneous immersion objective and Tolles's amplifier; spread and dried upon thin glass cover; heliostat.

FIG. 2. — BLOOD of yellow-fever patient, Havana, 1879 (fifth day of sickness, fatal case), magnified 1450 diameters by Zeiss's one-eighteenth-inch homogeneous immersion objective and Tolles's amplifier; spread and dried upon glass cover; heliostat.

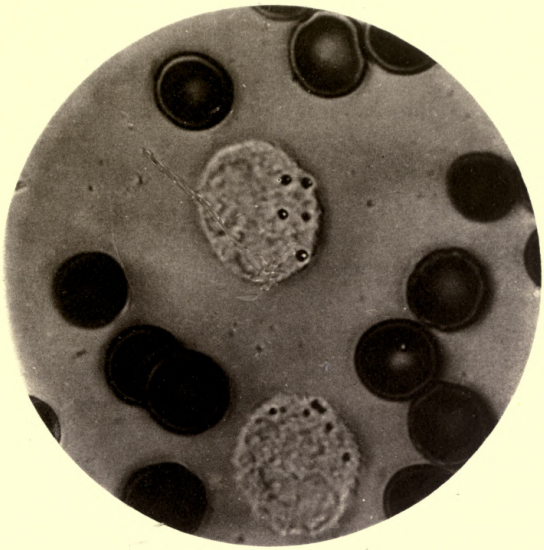


FIG. 1.

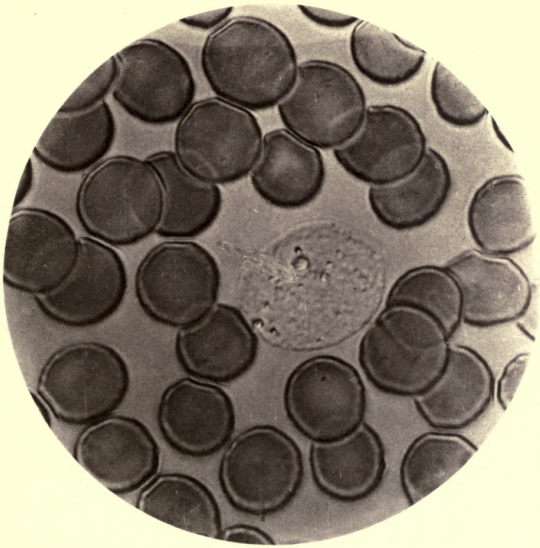


FIG. 2.

mation is produced by any cause, they do so in large numbers. Quite recently, the writer had the opportunity of seeing white blood-corpuscles in active amœboid movement, which had escaped from the capillary blood-vessels of an inflamed mucous membrane some hours previously. They were in urine, and had found their way into this fluid from the inflamed mucous membrane of the bladder. Under these circumstances, they pass under the name of pus-corpuscles; but their identity with the white corpuscles of the blood can scarcely be questioned.

If white blood-corpuscles or pus-corpuscles are spread upon a thin glass cover and allowed to dry, and are then treated with a suitable staining reagent, — such as methyl-violet, — it will be found that they are not homogeneous bits of protoplasm, but that they contain one or more deeply colored masses, called nuclei.

The white corpuscles are far more uniform in size than the red in different species of vertebrate animals, and do not vary greatly from $\frac{1}{3000}$ of an inch in the Mammalia.

The continued vitality of white blood-corpuscles and of ciliated epithelium cells after removal from the body of the animal in which they had their origin, is a striking phenomenon, because their spontaneous movements give evidence of this vitality; but there is no reason to suppose that these are exceptional cases. Indeed we have evidence that the vitality of detached tissues and even of organs may be preserved for a consid-

erable time. Thus portions of integument removed from one person or from an amputated limb may be transplanted to an open wound upon the surface of the body of another. Fingers which have been accidentally severed may be replaced, and sometimes become reattached. But the most remarkable example of this kind among warm-blooded animals is furnished by an experiment of Professor Martin, of Johns Hopkins University. This distinguished teacher and experimentalist has succeeded in keeping the heart of a dog alive for several hours after the body was dead. For this experiment the animal, deeply narcotized, is quickly dissected in such a manner that the heart and lungs are completely detached from the rest of the body, the large blood-vessels being ligated, except those which go to and from the lungs. Artificial respiration is kept up by means of bellows introduced into the trachea (wind-pipe), and the heart is supplied through a rubber tube with blood drawn from another animal. Under these conditions, its rhythmical contractions have continued for five hours.

In cold-blooded animals, the heart may continue to contract for hours when completely detached from the body, without any special precautions being taken to supply it with oxygenated blood.

These facts show that the popular idea that death of the whole body occurs at the instant that respiration ceases, "when the breath leaves the body," is fallacious.

For information in regard to the origin, structure, and functions of the blood-corpuscles, the reader is referred to standard works upon physiology and upon histology. Foster's "Physiology" may be especially recommended.

Pollen grains. (Plate VII.)

Pollen grains are living cells detached from the male organs of flowering plants, which are necessary for the fertilization of the ovule, which subsequently becomes a seed containing an embryo-plant. Like the spores of Fungi, they contain a portion of the protoplasm of the parent plant enclosed in a cell-wall, and, like these, they send out a slender filament when they are deposited in a locality suited for their germination. But, unlike these, they are incapable by themselves of producing a new plant, and they are even more particular than are the microscopic Fungi as regards the substratum upon which their germination may take place. This is limited to a great extent to the surface of the stigma of a plant of the species to which they belong. When so located, the pollen tube penetrates between the cells of the stigma, and, nourished by the juices of the female organ, extends through the whole length of the style to the ovule, — often a distance of an inch or more. Through this tube the fluid protoplasm, which contains numerous granules, passes, to be commingled with the protoplasm of the germ-cell.

PLATE VII.

FIG. 1. — POLLEN of *Lavatera ascergentifolia;* reflected light, San Francisco, 1882; magnified 25 diameters by Tolles's two-inch objective; illumination from blue sky (Lieberkühn).

FIG. 2. — POLLEN of *Acacia* (species ?); magnified 200 diameters; Zeiss's one-sixth-inch objective (dry); mounted in glycerine; no heliostat.

FIG. 3. — POLLEN of *Pine* (species ?); magnified 75 diameters by Beck's two-thirds-inch objective; mounted in glycerine; no heliostat.

FIG. 4. — POLLEN of *Azalea* (species ?); magnified 75 diameters by Beck's two-thirds-inch objective; mounted in glycerine; no heliostat.

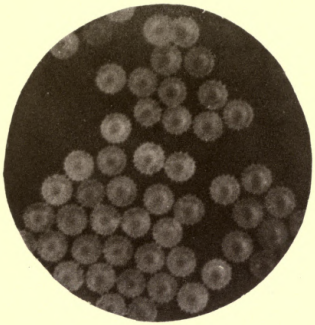


FIG. 1.

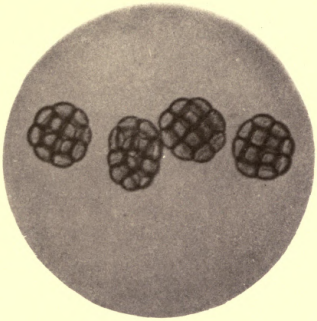


FIG. 2.

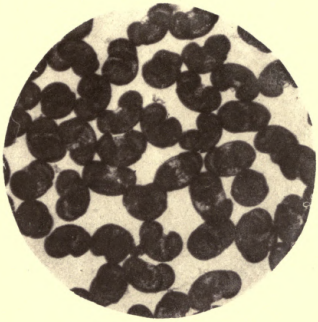


FIG. 3.

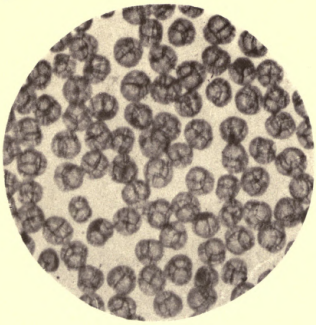


FIG. 4.

The result of this "conjugation" has already been referred to.

Pollen grains are formed in the anthers of plants in "mother cells," by segmentation of the contained protoplasm (endoplasm) of these cells into four masses, which subsequently are invested with a double cellular envelope. The external envelope is comparatively thick, and in many species is covered with peculiar markings or with little spines, which make these pollen grains very curious and interesting objects for microscopical study, aside from the interest which attaches to them on account of the part which they play in the reproduction of the plant.

For the benefit of those who have a microscope at hand, and who have been at a loss for interesting objects to examine, a few of the plants having especially curious pollen grains may be mentioned: pollen of daisy (*Bellis perennis*); of passion-flower (*Passiflora cœrulea*); of musk-plant (*Mimulus moschatus*); of *Althœa rosea;* of pumpkin (*Cucurbita pepo*); of *Cobœa scandens;* of *Ipomœa purpurea;* of chicory (*Cichorium intybus*). The compound pollen masses of the different species of Acacia are extremely interesting, and are characteristic of the genus (see Plate VII. Fig. 2). In Œnothera, the grains have a triangular outline, and in the Malvaceæ, they are spherical, and covered with short spines.

The curious bi-lobate pollen grains of the pine are seen in Plate VII. Fig. 3. In the city of New Orleans, after a rainfall in the spring of

the year, a yellow streak is often left upon the surface of the moist earth along the margins of the gutters. Upon microscopical examination, this will be found to be made up of these bilobate pollen grains from the pine which have been washed from the atmosphere by the falling rain. There are, however, no pine forests in the immediate vicinity, and the pollen is evidently transported many miles by the winds. This fact illustrates the bountiful supply of these reproductive elements provided by nature, and also furnishes an important hint to sanitarians. If pollen grains can be carried such long distances, we may be sure that the minute organisms and their germs (spores) which find their way into the atmosphere from all sorts of foul hotbeds may also be transported in the same way, and that they too are washed out by the falling rain. So that rain-water, commonly considered the purest water to be had for drinking, is in reality "the sewage of the atmosphere," as it has been aptly called by a distinguished surgeon of the army. The sanitary lessons to be drawn from these facts are evident. Preserve the atmosphere from contamination by destroying filth — organic matter undergoing decomposition — by covering or draining exposed mud-flats, etc., and reject the rain-water which first falls when there is reason to believe that the atmosphere is contaminated with these germs of putrefaction or of disease — during the summer months, especially in cities, and during the prevalence of an epidemic.

For fuller information regarding pollen, the student is referred to the Micrographic Dictionary.

Epidermis of Plants. (Plate VIII.)

The higher plants are invested with a membrane made up of a mosaic of more or less flattened cells, which is called the epidermis. In many plants this may be peeled off from the green twigs or from the surface of the leaves. The examination of such a specimen under the microscope is interesting not only on account of the beauty and variety of patterns exhibited in this natural mosaic, but also as a lesson in vegetable histology.

The cells of the epidermis are usually colorless, and being joined by their margins in a single plane, no preparation is required except to place a fragment of proper size in water or glycerine under a thin glass cover. They are readily seen with a low-power objective, — one inch or one-half inch, — and usually a flattened *nucleus*, containing a *nucleolus*, may be seen attached to one portion of the cell wall.

Detached cells from the parenchyma of the leaf containing a quantity of green granules (chlorophyl) are sometimes seen adhering to the fragment of epidermis which has been stripped from a succulent leaf or green twig.

Plate VIII. Figs. 1, 2, 3, and 4, will serve to give an idea of the variety of patterns resulting

PLATE VIII.

FIG. 1. — EPIDERMIS of *Lilium longiflora*, magnified 50 diameters by Collins's one-inch objective; not stained; no heliostat.

FIG. 2. — EPIDERMIS of Anagalis, San Francisco, 1882; magnified 50 diameters by Beck's two-thirds-inch objective; not stained; no heliostat.

FIG. 3. — EPIDERMIS of Echeveria, San Francisco, 1882; magnified 75 diameters by Beck's two-thirds-inch objective; not stained; no heliostat.

FIG. 4. — EPIDERMIS of *Agave Americana*, San Francisco, 1882; magnified one hundred diameters; Collins's one-inch objective; no heliostat.

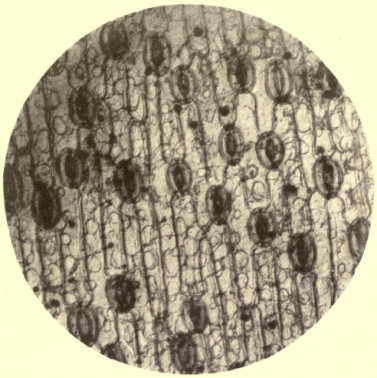


FIG. 1.

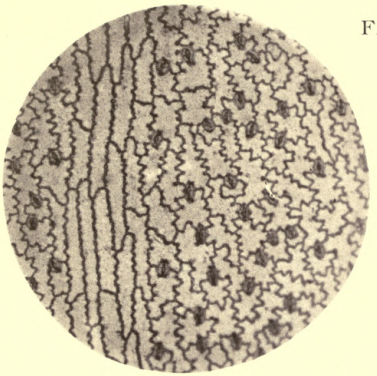


FIG. 2.

FIG. 3.

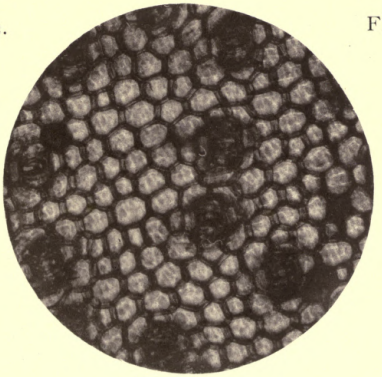


FIG. 4.

from the different forms and various modes of grouping of these epidermal cells, and of the beautiful arrangement, for the end in view, of the cells of the stomata, or breathing-pores. These stomata are openings through which air is admitted to the lungs (leaves) of the plant. On each side of the stoma, in most plants, there is a semilunar "guard-cell." These are well seen in Fig. 1. The office of this pair of cells seems to be to regulate the admission of air through the stoma according to the requirements of the plant, and with reference to the hygrometric condition of the atmosphere. Evidently the stomata (apertures) will be of greater or less size according as the guard-cells are distended or otherwise with fluid. The stomata are mostly found upon the lower surface of leaves, although in some species they are also abundant upon the upper surface as well.

Their number has been estimated, by careful counting, for a number of species, and is given as 160,000 per square inch in the lilac; 63,000 in the holly; 2,000 in Tradescantia; 200 in the mistletoe, etc. The reader interested in vegetable histology and physiology, will do well to obtain a copy of Professor Sachs' "Text-book of Botany" (English translation by Bennett).

PLATE IX.

FIG. 1. — TRANSVERSE SECTION OF PETIOLE of *Calla*, San Francisco, 1882; magnified 60 diameters by Collins's one-inch objective; mounted in water; no heliostat.

FIG. 2. — STELLATE HAIRS on a leaf of *Deutzia scabra*, San Francisco, 1882: Reflected light (Lieberkühn); magnified 40 diameters by Tolles's two-inch objective; mounted dry; no heliostat.

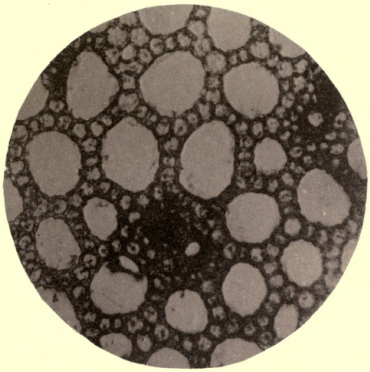


FIG. 1.

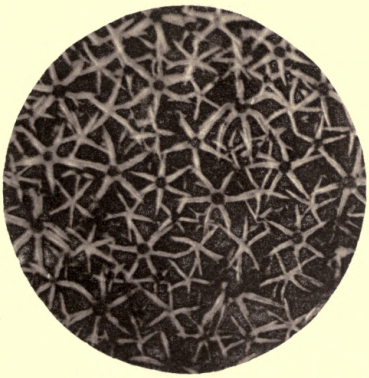


FIG. 2.

Plant Hairs. (Plate IX. Fig. 2.)

The surface of the leaves of certain plants presents a glistening appearance (*e. g.*, ice-plant), due to the presence of little elevated transparent vescicles which appear like drops of dew attached to the leaves. Under a low magnifying power it will be seen that these are simply cells of the epidermis which are elevated above the general level to form little hemispherical projections, which, being filled with fluid and having a transparent cell-wall, glisten like dewdrops.

Again, instead of a hemispherical projection, we may have a little cone, as in the cabbage leaf. This is a plant hair, and in its simplest form it is nothing more than an elongated cell of the epidermis. But nature does not confine herself to simple and stereotyped forms, seeming rather to delight in variety and even eccentricity in the moulding of the cellular elements out of which plants and animals are built. Thus these plant hairs may be composed of a single cell having a spherical (calyx of Salvia) or bulbous extremity; or of a cell enormously elongated; or of a cell divided at its extremity into two or more branches (Draba, Allysum). Again, they may be composed of a linear series of elongated or spherical cells (stamens of Tradescantia). The typical cell is spherical, and we can easily account, under mechanical laws, for changes in form resulting from pressure, as in the pith of plants, etc. But what is the

moulding power which causes these plant hairs, arising from the free surface of the leaves of different species of plants, to assume such varied forms? And how is it that this moulding power always produces little stars upon the leaf of one species — *e. g.*, *Deutzia scabra* — and simple unbranched hairs upon another — *e. g.*, Brassica? This is a fundamental question in biology, and one that is only vaguely answered by science in the statement that it is in accordance with the laws of heredity. The case is of the same nature, although vastly more complicated in the outcome, as among the unicellular organisms, the simple life-history of which has already been detailed. One little mass of protoplasm enclosed in a cellular envelope grows by appropriating to itself the necessary material from the nutritive medium in which it is placed, and multiplies by binary division, producing only little spheres (Micrococci), which, however, present noticeable differences as to dimensions. Another similar mass has an elongated form (Bacilli) which is preserved in its progeny; but these also present slight variations as to length and diameter. A third species presents a spiral form, which is preserved from generation to generation. In the interior of these rods or spiral filaments, spherical masses of protoplasm (spores) are formed which cannot be distinguished one from another by the highest powers of the microscope, but which are nevertheless *potentially different*, for one in growing produces a rod, and the other a spiral filament.

So in higher plants, the peculiar structures under consideration (plant hairs) owe their form to a moulding power residing in the living protoplasm contained in their cellular envelope, which is derived from the little mass of protoplasm detached from the parent — the fertilized ovule — in which the potentiality which has resulted in this wonderful development was at one time stored up. But this hereditary transmission of ancestral features does not result in tame uniformity. While in a general way plants and animals resemble their parents, there is sufficient variation in detail to make every individual different in some respect from others of the same species. Thus, among plant hairs we may find upon the surface of the same leaf various modifications of the characteristic form. Upon the leaf of *Lavatera ascergentifolia*, for example, the stellate hairs sometimes have but two or three rays, while others in the same field of view may have as many as seven or eight.

The phenomenon of *cyclosis*, or rotation of the protoplasm within the cell, may be favorably studied in the hairs of certain plants, *e. g.*, in Tradescantia.

It will be seen from what has been said, that plant hairs are interesting objects of study, not only for the amateur microscopist who seeks curious and beautiful objects for his cabinet, but also for the biologist and the student of vegetable physiology.

PLATE X.

FIG. 1. — TRANSVERSE SECTION OF LEAF of pampas-grass (*Gynerium argenteum*), San Francisco, 1882, magnified 40 diameters by Collins's one-inch objective; mounted in glycerine; no heliostat.

FIG. 2. — TRANSVERSE SECTION OF LEAF of pine, San Francisco, 1882, magnified 40 diameters by Collins's one-inch objective; no heliostat.

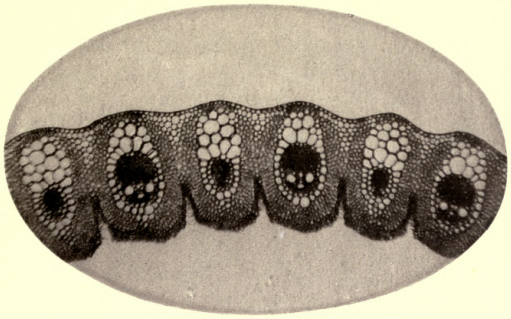


FIG. 1.

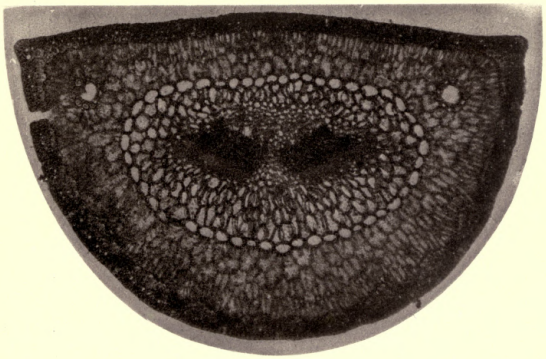


FIG. 2.

SECTIONS OF VEGETABLE TISSSUES (PLATE IX. FIG. 1; PLATE X. FIGS. 1 AND 2; AND PLATE XI. FIGS. 1 AND 2).

In the epidermis of plants, we have had an example of the methods in which flattened cells are joined side by side to form an investing membrane for the higher plants. We have now to glance hastily at the interior structure of these, and we shall find that here also we have uniformity in plan and diversity in execution. With cells of various forms and dimensions as the architectural units, we have formed a variety of vegetable tissues which serve various purposes in the economy of the plants of which they are a part.

In a work of this kind, anything more than the merest outline with regard to this subject would be out of place, and the reader whose curiosity is aroused by an inspection of the photo-micrographs (Plates IX., X., and XI.) in the present volume, can easily obtain detailed information from standard botanical text-books.

A very beautiful example of cell-masonry is seen in Plate IX. Fig. 1, which is from a transverse section through the petiole of Calla. Here the cells of the pith are built up into partitions enclosing cavities, and we have thus formed a structure having considerable strength, with a comparatively small expenditure of material. Instead of partition walls, we sometimes have, in endogenous stems, cavities, the walls of which are

braced apart by stellate cells having long arms, the extremities of which meet in the interior. This structure may be seen in Canna. The pith of the rush and of numerous water-plants is also made up of stellate cells.

The difference in the structure of *Exogens* and *Endogens* is illustrated in Plate XI. In the one case (Exogens, Fig. 1), the interior of the stem is made up of a loose cellular structure, *pith;* next to this comes the woody part of the stem, containing the *fibro-vascular bundles;* and outside of all, the cells making up the bark. In the Endogens, on the contrary, there is no distinct wood, pith, and bark, and the fibro-vascular bundles are scattered through the stem. These are seen in cross-section in Fig. 2, but a longitudinal section is required to show their structure, and, indeed, many sections from various plants will be necessary to illustrate this in a satisfactory manner.

In Plate X., Fig. 1, we have a picture which might easily pass for a pattern of lace-work, and one might wonder at the odd conceit of the person who designed it in introducing a figure of a skull into every second scallop. This design, however, is made by Dame Nature, as the photo-micrograph is from a thin transverse section of the leaf of Pampas-grass.

DIATOMS. (PLATES XII., XIII., XIV., XV. AND XVI.)

Diatoms are unicellular vegetable organisms which abound in fresh, brackish, and salt water, where they are usually to be found adhering to stones, to various water-plants, or to decaying vegetable material. They belong to the Algæ, and contain a coloring matter which appears to be a modification of chlorophyl, but which has a yellowish-brown color instead of the bright green hue which this pigment has in higher plants. This is in minute granules, or is diffused through the cell contents — *endochrome*. The peculiarity of diatoms which gives them a special interest for microscopists, consists in the presence of a rigid casing of silex, which encloses the cells. This consists of two *valves*, which are usually more or less convex, and between which the living protoplasm with its cellular envelope is enclosed, very much as a clam or any other bivalve mollusk is enclosed in its shell. But the siliceous shell of the diatom is transparent, and permits us to see plainly the *endochrome* within. These siliceous valves are the "diatoms" with which microscopists are familiar in mounted preparations, and they will always be favorite objects, both on account of the beauty and variety of forms which they present, and because the delicate markings upon some of them are used as tests of the resolving power of objectives.

These markings are in the form of bands, lines, dots, etc. They can seldom be distinguished in

PLATE XI.

FIG. 1. — TRANSVERSE SECTION OF STEM of *Abutilon striatum*, San Francisco, 1882; magnified 40 diameters by Collins's one-inch objective; mounted in glycerine; no heliostat.

FIG. 2. — TRANSVERSE SECTION OF STEM of *Canna Indica*, San Francisco, 1882; magnified 40 diameters by Collins's one-inch objective; mounted in glycerine; no heliostat.

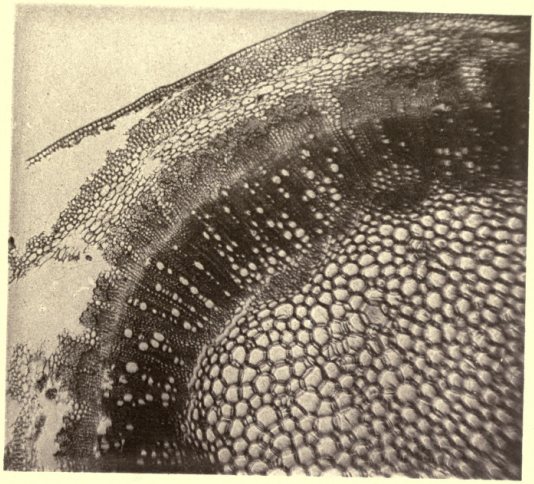


FIG. 1.

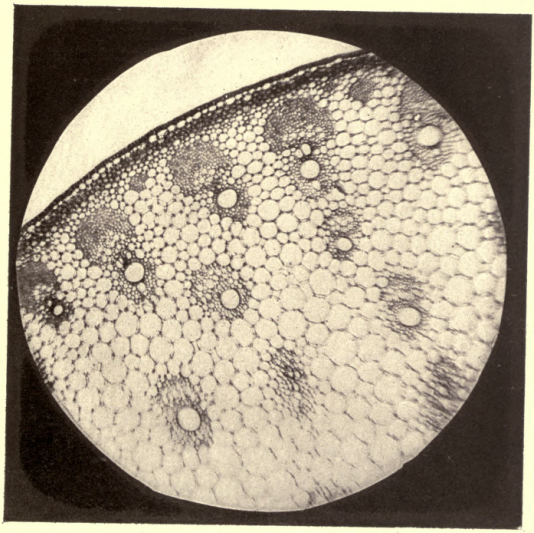


FIG. 2.

the living diatom, and it is only when the valves have been properly prepared by freeing them from all organic matter that they are distinctly seen. In the more difficult test-diatoms these markings are so minute that they are only brought into view by the use of wide-angled objectives of high power and by skilful manipulation of the light, — oblique illumination.

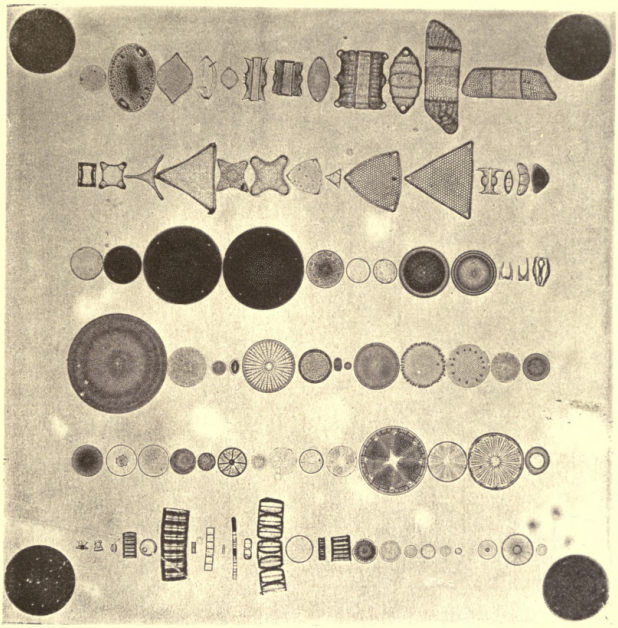
The mode of preparation for living diatoms consists in the destruction of the organic material — cell-wall and contents — by means of cineration or by boiling in strong nitric acid. In the fossil forms, this destruction of the organic portion of the little plant has been accomplished by "the hand of time."

In consequence of this indestructible siliceous shell, a record is left to us of the diatomaceous flora of past ages, and while countless generations of unicellular Algæ, and of Infusoria which were associated with them in the ponds and streams of the past, have disappeared without leaving a trace of their existence, we have abundant material for the study of fossil diatoms.

One of the most famous localities, both on account of its extent and the variety of species found, is in the vicinity of Richmond, Va. This is a marine deposit which underlies the city, and extends for an unknown distance around it. The thickness of this deposit is said to be about eighteen feet. When we consider the minute size of these exquisite little natural gems, and the conditions under which they are formed, we

PLATE XII.

FOURTH SQUARE of MÖLLER'S DIATOMACEEN TYPEN-PLATTE (Type-plate of the *Diatomaceæ*); magnified 50 diameters by Powell & Lealand's one-half-inch objective (New Orleans, 1880); heliostat.



cannot fail to be impressed with the immense period of time which must have been required for the formation of a deposit of this thickness.

The plates introduced into the present volume will serve to illustrate the beauty and variety of forms presented by these interesting objects.

In Plate XII. we have, arranged in six rows, ninety of these siliceous valves from various parts of the world, and including both recent and fossil species. The amplification is not sufficient (fifty diameters) to show the more delicate markings, and, indeed, some of the smaller species are only imperfectly seen (*vide* page 22). After admiring the beauty of these diatoms in detail, the reader cannot fail to be astonished at the skill of the preparator who has selected them from rough material collected in distant parts of the globe, and has arranged them in this methodical manner, not only giving each its proper place in the *Typen-Platte*, but excluding all extraneous particles — dust — which would mar the beauty of the picture.

This is only one of four squares all arranged in the same way and upon a single slide, where the space occupied is less than one-sixth of an inch in area, while the number of diatoms is 472.

Some amateur microscopists in this country have succeeded in arranging a smaller number of diatoms in regular order in a very creditable manner; but the patience and technical skill exhibited in the preparation of the *Typen-Platte* of Möller are not likely to be equalled, and can scarcely be excelled.

PLATE XIII.

TRICERATIUM FAVUS, San Francisco, 1882; magnified 400 diameters by Tolles's four-tenths-inch objective and amplifier; heliostat.

FIG. 1. — Centre in focus.

FIG. 2. — Margin in focus.

FIG. 1.

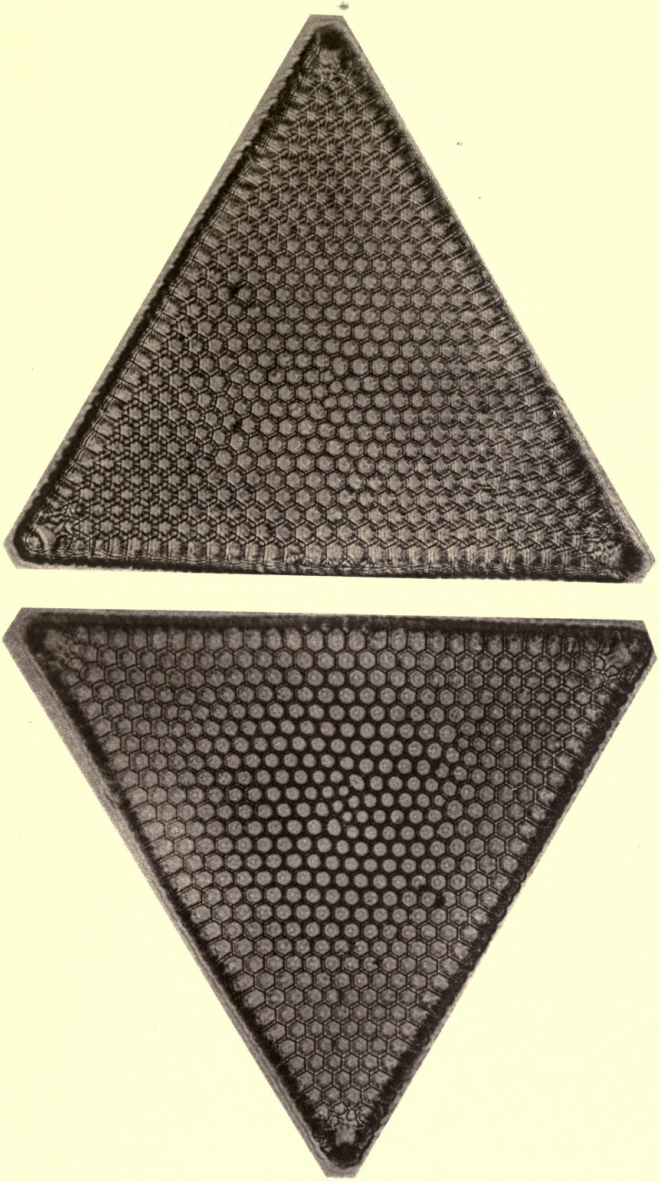


FIG. 2.

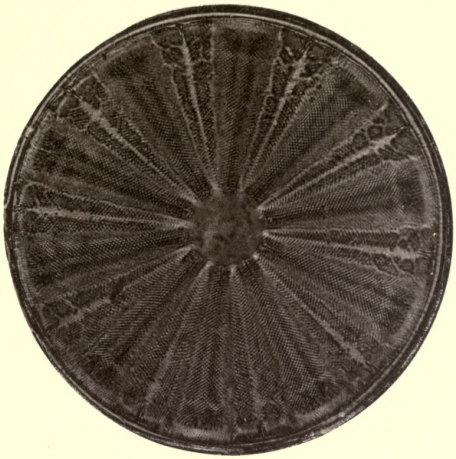


FIG. 1.

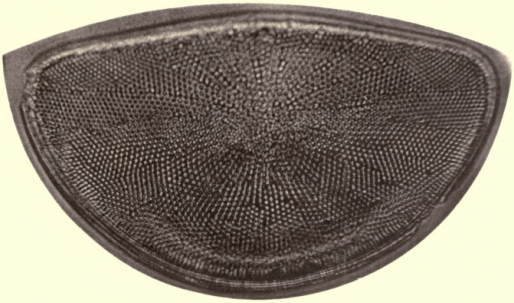


FIG. 2.

PLATE XIV.

FIG. 1. — ACTINOPTYCHUS HALIONYX, New Orleans, 1880; magnified 375 diameters by Spencer's one-sixth-inch glycerine immersion objective; heliostat.

FIG. 2. — EUODIA GIBBA, New Orleans, 1880; magnified 500 diameters by Spencer's one-tenth-inch glycerine immersion objective; heliostat.

PLATE XV.

FIG. 1. — COSCINODISCUS OCULUS IRIDIS, by dark-ground illumination, San Francisco, 1882; magnified 200 diameters by Tolles's four-tenths-inch objective; heliostat.

FIG. 2. — The same diatom (*Coscinodiscus Oculus Iridis*), New Orleans, 1880; magnified 300 diameters by Spencer's one-sixth-inch glycerine immersion objective; heliostat.

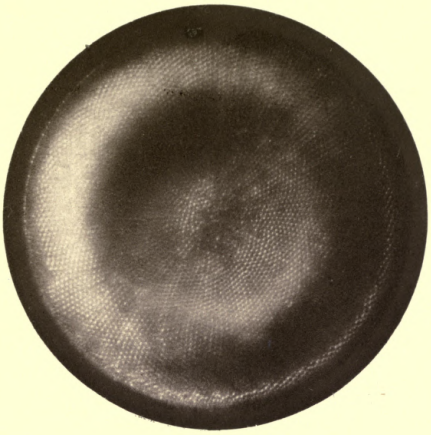


FIG. 1.

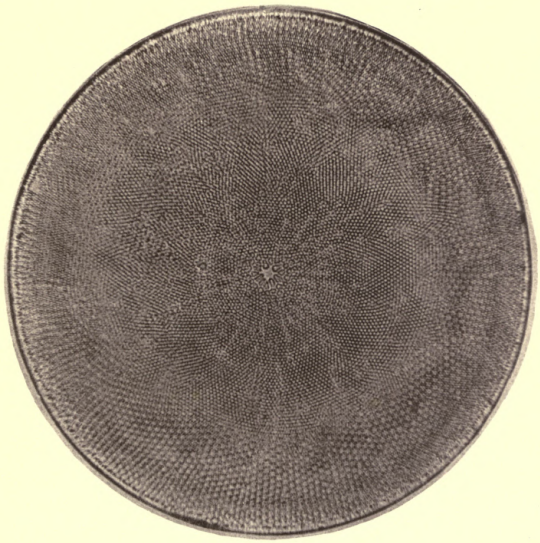


FIG. 2.

The preparation and mounting of mixed diatoms in balsam is not so difficult a matter, and every microscopist should make himself familiar with the technique, which is given in the Microscopic Dictionary and in Dr. Carpenter's admirable work, which has already been frequently referred to.

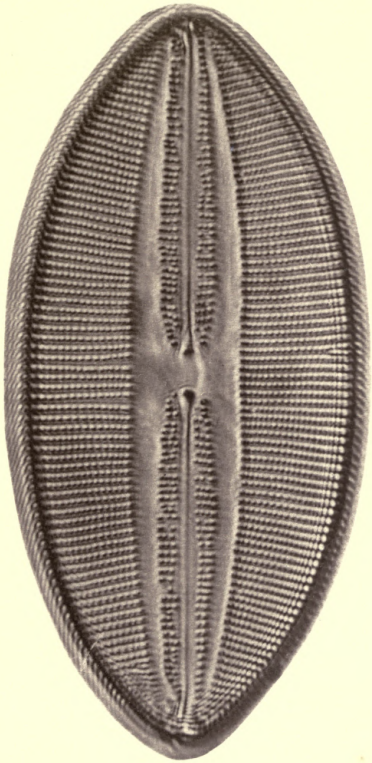
The fascinating work of collecting and mounting cabinet specimens of the silicified valves commonly known as diatoms, should not occupy the attention of the amateur microscopist to the exclusion of the study of the life-history of the unicellular plants which they enclose.

The usual mode of increase is the same as among other unicellular organisms : viz., by binary division. This occurs in a plane parallel with the flat surfaces of the two valves, by a division of the cell contents, and the formation of a double membranous partition, in each wall of which silex is deposited to form a new valve. Two individuals are thus formed like the parent-form, which when separated have each one old and one new valve.

Conjugation also occurs among the Diatomaceæ. We have already seen that this curious mode of reproduction is common among unicellular organisms. In the case of diatoms, it occurs between two cells —*frustules* — which, lying in proximity, open at the margin, —*suture*, — and permit of the extrusion of the cell-contents, which coalesce and form a new frustule. This becomes invested with a silicified casing, like that

PLATE XVI.

NAVICULA LYRA, San Francisco, 1882; magnified 800 diameters by Zeiss's one-sixth-inch objective (dry) and Tolles's amplifier; heliostat.



of the parent-form, but necessarily on a larger scale, as it contains the material which before made up two individuals. These larger diatoms, resulting from conjugation, multiply again by binary division, and in the course of a number of generations the size is diminished to that of the individual members of the conjugating pair. It will be seen that in these marriages in low life, the twain literally become one flesh.

List of Diatoms in Plate XII.

Abbreviations used: F. W., fresh water; M., marine; B. W., brackish water; V., valve; U. V., upper valve; L. V., lower valve; P. V., principal view.

First Line.

No.	1.	Isthmia enervis.	M.	*North Sea.*
"	2.	" nervosa.	M.	*California.*
"	3.	Biddulphia pulchella.	V., M.	*Tongatuba.*
"	4.	" "	P. V.	"
"	5.	" Roperiana.	V., M.	*Australia.*
"	6.	" "	P. V.	"
"	7.	" Aurita.		*Peru-Guano.*
"	8.	" reticulata.	M.	*Australia.*
"	9.	" Baileyii.	M.	*Cuxhaven.*
"	10.	" Rhombus.	M.	*Cuxhaven.*
"	11.	Cerataulus turgidus.	M.	*Australia.*
"	12.	" Smithii.	M.	*Cuxhaven.*

Second Line.

No.	1.	Euodia gibba.		*Fossil. Spain.*
"	2.	" Frauenfeldii.	M.	*Australia.*
"	3.	Corinna elegans.	V.	*Fossil. Jutland.*
"	4.	" "	P. V.	
"	5.	Triceratium favus.	M.	*Holstein.*
"	6.	" fimbriatum.	M.	*Australia.*

No. 7.	Triceratium alternans.		*Peru-Guano.*
" 8.	" striolatum.	M.	*Cuxhaven.*
" 9.	" antedeluvianum.	M.	*Holstein.*
" 10.	" pentacrinus.	M.	*Australia.*
" 11.	Trinacria regina.		*Fossil. Jutland.*
" 12.	" excavata.		*Fossil. Jutland.*
" 13.	Solium exculptum.	V.	*Fossil. Jutland.*
" 14.	" "	P. V.	

Third Line.

No. 1.	Goniothecium Danicum.		*Fossil. Jutland.*
" 2.	Hemiaulus polycystinorum.		*Fossil. Barbadoes.*
" 3.	" alatus.		*Fossil. Barbadoes.*
" 4.	Actinocyclus Ralfsii.	M.	*Cuxhaven.*
" 5.	" dubius.		*Fossil. Australia.*
" 6.	Auliscus sculptus.	M.	*Cuxhaven.*
" 7.	" macræanus.	M.	*Australia.*
" 8.	Aulacodiscus crux.		*Peru-Guano.*
" 9.	Eupodiscus Argus. Var. 5 app.	M.	*Cuxhaven.*
" 10.	" " " 4 app.		
" 11.	" " " 3 appendages.		
" 12.	" radiatus.	M.	*S. America.*

Fourth Line.

No. 1.	Odontodiscus subtilis.	B. W.	*Holstein.*
" 2.	" eccentricus.	M.	*Holstein.*
" 3.	Systephania diadema.		*Fossil. N.-America.*
" 4.	" corona.		*Fossil. N.-America.*
" 5.	Peristephania eutycha.		*Fossil. Spain.*
" 6.	Stephanopyxis appendiculata.	V.	*Fossil. Jutland.*
" 7.	" "	P. V.	
" 8.	Endyctia oceanica.		*Peru-Guano.*
" 9.	Arachnoidiscus ornatus.	M.	*Japan.*
" 10.	Cestodiscus ovalis.		*Fossil. Spain.*
" 11.	Coscinodiscus minor. (var.)		*Fossil. Nankoori.*
" 12.	" radiatus.	M.	*Holstein.*
" 13.	" oculus iridis.		*Fossil. Jutland.*

Fifth Line.

No.	1.	Craspepodiscus coscinodiscus.		*Fossil. Nankoori.*
"	2.	Actinoptychus halionyx.		*Peru-Guano.*
"	3.	" splendens.	M.	*Holstein.*
"	4.	" heliopelta.		*Fossil. N. America.*
"	5.	" undulatus.	M.	*Holstein.*
"	6.	" areolatus.		*Peru-Guano.*
"	7.	Asteromphalus Ralfsianus.		*Peru-Guano.*
"	8.	" Arachne.		*Peru-Guano.*
"	9.	Stephanagonia Danica.		*Fossil. Jutland.*
"	10.	Stictodiscus angulatus.		*Fossil. Jutland.*
"	11.	Pyxidicula cruciata.		*Fossil. Jutland.*
"	12.	Hyalodiscus stelliger.	M.	*California.*
"	13,	" subtilis.	M.	*California.*
"	14.	Symbolophora Trinitatis (var.).		*Fossil. Jutland.*

Sixth Line.

No.	1.	Discoplea Sinensis (var.).		*Fossil. Australia.*
"	2.	Podosira maculata.	M.	*Holstein.*
"	3.	" nummuloides.	M.	*Australia.*
"	4.	Cyclotella Meneghiniana.	F. W.	*Holstein.*
"	5.	" minutula.		*Fossil. Lüneberg.*
"	6.	" affinis.		*Fossil. N. America.*
"	7.	" striata.	B. W.	*Holstein.*
"	8.	" operculata.	M.	*Fossil. Hanover.*
"	9.	" Astrea.		*Fossil. Lüneberg.*
"	10.	" " (var.)		*Fossil. California.*
"	11.	" radiata.	M.	*California.*
"	12.	Orthosira solida.		*Fossil. California.*
"	13.	Melosira Borrerii.	V., M.	*Schleswig.*
"	14.	" "	P. V.	
"	15.	" nummuloides.	M.	
"	16.	" crenulata.	F. W.	*Holstein.*
"	17.	" distans.		*Fossil. Bohemia.*
"	18.	" varians.	F. W.	*Holstein.*
"	19.	" punctata.		*Fossil. Hanover.*
"	20.	" arenaria.	F. W.	

PLATE XVII.

FIG. 1. — ADIPOSE TISSUE OF OX, San Francisco, 1882; magnified 50 diameters by Beck's two-thirds-inch objective; mounted in glycerine; no heliostat.

FIG. 2. — ROUND-CELLED SARCOMA, San Francisco, 1882; magnified 100 diameters by Tolles's four-tenths-inch objective; mounted in balsam; carmine-stained; no heliostat.

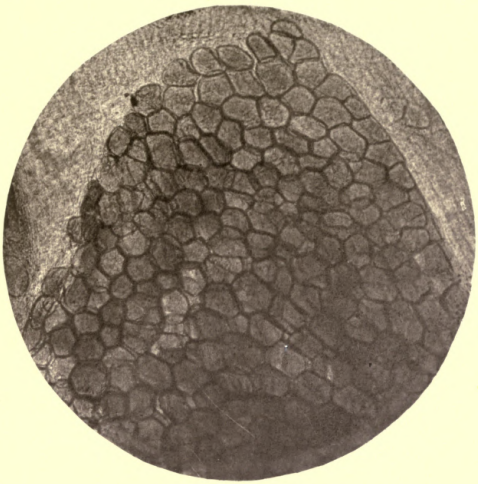


FIG. 1.

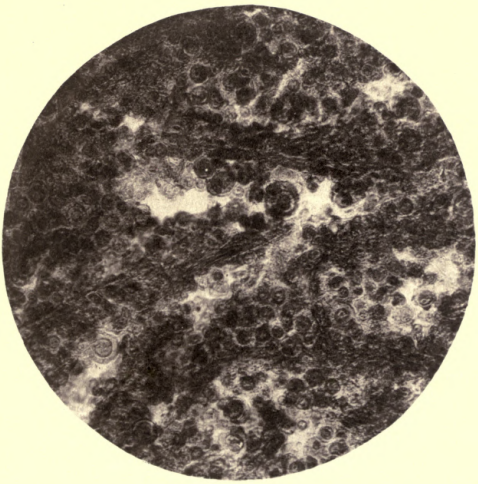


FIG. 2.

No. 21.	Melosira sulcata.	V.	*Peru-Guano.*
" 22.	" "	P. V.	
" 23.	Chætoceras didymum.		*Peru-Guano.*
" 24.	Dicladia capreolus.		*Peru-Guano.*
" 25.	Syndendrium diadema.		*Peru-Guano.*

Sections of Animal Tissues.

The fact that vegetable tissues are built up of *cells* has been amply illustrated in the present volume. The same is true of animal tissues, and in some of these they may be readily demonstrated, as for example, in adipose tissue (Plate XVII. Fig. 1).

In other cases, the cells being much smaller and the investing membrane extremely delicate, the demonstration can only be made by resorting to special methods of preparation, and especially to staining processes. Thus the delicate membrane which lines the blood-vessels appears to be homogeneous when examined in water or glycerine without staining; but the outlines of the separate cells of which it is made up are brought into view when the preparation is stained with nitrate of silver. Again, there may be a wide departure from the form of the typical cell, as in muscular fibres, which are greatly elongated fusiform cells.

A knowledge of the details of animal histology and of the various methods of preparing animal tissues for microscopical examination may be obtained from the works of Frey, Beale, Stricker, and Satterthwaite, already referred to (pp. 118, 119).

An excessive development of the cells of a part or an infiltration of foreign cells among the normal histological elements results in the formation of a tumor — non-malignant, as in fatty tumors, etc., or malignant, as in the varieties of cancer.

We have an example of this in Plate XVII. Fig. 2, in which spherical nucleated cells are to be seen infiltrated among the tissues (the female breast), constituting the malignant disease known as sarcoma, a variety of cancer.

Parasites. Plates XVIII. and XIX.

Microscopists have given much time and research to the study of the parasitic fauna and flora of plants and animals. But the field is so broad that there is still much to be learned in this direction. The larger parasites, such as are seen in our Plates XVIII. and XIX., have long been known, and, indeed, some of them may be readily seen with the naked eye.

These more conspicuous animal parasites belonging to the class Insecta (Plate XVIII. Figs. 1 and 2) and to the class Arachnida, family Acarina (Plate XIX. Figs. 2 and 3), although they furnish a large number of species, nearly every bird and mammal being infested with one or more species peculiar to it, have less practical interest for *civilized* man than have the Entozoa (internal parasites) and the parasitic Fungi, against the attacks of which he finds it more difficult to defend himself.

With the advance of civilization and of science the first-named parasites have been banished from good society; the primitive methods of limiting their numbers resorted to by our savage ancestors, and by the lower animals generally, which may still be witnessed in the wigwam of an American Indian or in the monkey-cage of any zoölogical collection, having given way to personal cleanliness and to the use of parasiticides.

The larger parasites, which live upon the surface or burrow a short distance into the skin (acari), although extremely annoying to their hosts, do not endanger life or materially interfere with the health of the individual. But recent researches have shown that there are parasites very much smaller and lower in the scale of life which may invade the blood or tissues of their host in such vast numbers as to interfere with vital processes and produce disease of the most fatal character. The disease of silk-worms, *pébrine*, so successfully studied by Pasteur, is of this character. Anthrax (*charbon* of the French), a disease of sheep and cattle which may also be communicated by inoculation to man, is another example. Other examples might be given, and there is good reason for believing that all the infectious diseases of man and of the lower animals will eventually be proved to be parasitic. Evidently this would be a demonstration of the greatest importance to mankind, and if once fairly established, it would doubtless lead to improved methods of combating these diseases.

PLATE XVIII.

FIG. 1. — PARASITE of greyhound, magnified 35 diameters by Tolles's two-inch objective ; mounted in balsam ; heliostat.

FIG. 2. — PEDICULUS VESTMENTI, magnified 12 diameters by Tolles's two-inch objective ; mounted in balsam ; heliostat.

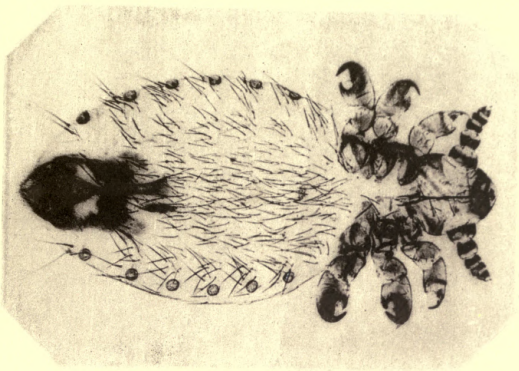


FIG. 1.

FIG. 2.

Even if we do not discover means of destroying these disease-producing parasites within the body of the sick person invaded by them, we shall know where to find them and how to fight them in their external habitats; and by destroying the germs of disease in the discharges of those sick with cholera and typhoid fever, in the expectoration of those suffering from diphtheria and consumption, etc., we can, no doubt, greatly limit the ravages of these diseases. Indeed, it is not too much to predict that the time will come when epidemics of these preventible parasitic diseases will be considered as great a reproach to a community as the vulgar external parasites of man are to the individual infested with them among enlightened people.

The writer would not be understood as affirming that the diseases mentioned have all been proved to be parasitic. There is much evidence in favor of this view, and in the case of several of the infectious diseases the demonstration is complete. But satisfactory proof is still wanting as regards the greater number, and it would be unscientific to generalize in the present state of investigation. The question is now being studied by the experimental method by microscopical experts in various parts of the world. Germany and France have taken the lead in this line of investigation, and Government aid has been freely extended to those investigators who have contributed the most to our knowledge of "disease-germs." Unfortunately the enlightened policy of the French and German

PLATE XIX.

FIG. 1. — TICK of hedgehog, magnified 12 diameters by Tolles's two-inch objective ; in balsam ; heliostat.

FIG. 2. — PARASITE of beetle, magnified 12 diameters ; Tolles's two-inch objective ; in balsam ; heliostat.

FIG. 3. — ACARUS of housefly, magnified 100 diameters ; Collins's one-inch objective ; heliostat.

FIG. 4. — PARASITE of bat, magnified 50 diameters by Tolles's two-inch objective ; in balsam ; heliostat.

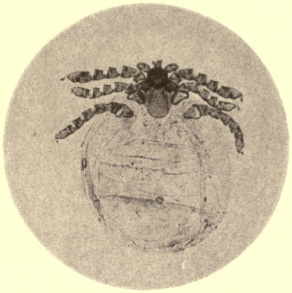


FIG. 1,

FIG. 2.

FIG. 3.

FIG. 4.

Governments, by which scientific research has been stimulated and fostered, has not controlled our national legislators, and America has contributed but little to the advance of scientific knowledge in this direction. These researches present very great difficulties, owing to the minute size of the micro-organisms which are believed to be the cause of the diseases in question, and because of the multitude of harmless parasites of the same class (bacteria) which constantly infest the alimentary canal of man and of the lower animals. These quickly invade the blood and the tissues after death, and possibly before death, and have frequently been described by untrained observers as veritable disease-germs. This has led to great confusion and to contradictory statements from different sources, which have done much to retard progress, and have given some foundation for the ridicule with which certain well-meaning but only partially informed physicians are disposed to treat the whole subject. In view of these facts, investigators in this field agree that it is desirable to make photo-micrographs of these minute organisms, in order that slight morphological differences may be detected. These would also assist greatly in the classification of the parasitic bacterial flora of plants and animals and that of organic infusions to which floating atmospheric germs have access.

PLATE XX.

FIG. 1. — TEETH upon surface of tongue of snail (*Helix pomatia*); magnified 80 diameters by Collins's one-inch objective; in balsam; heliostat.

FIG. 2. — TRACHEÆ of silkworm, magnified 15 diameters by Tolles's 2-inch objective; in balsam; heliostat.

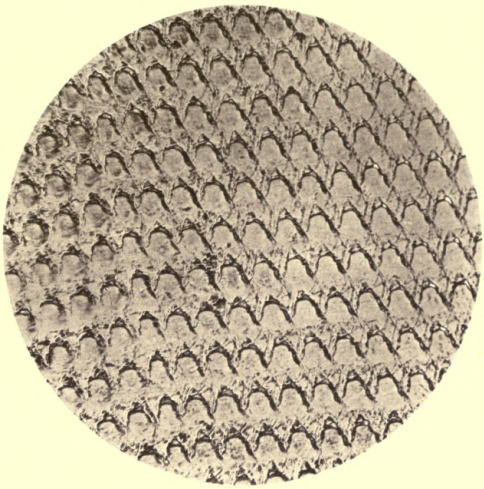


FIG. 1.

FIG. 2.

Teeth of Mollusca. Plate XX. Fig. 1.

By careful dissection, the student will be able to obtain from the Mollusca a variety of interesting objects for study under the microscope. Most attractive and curious among these are the teeth upon the lingual membrane of these creatures. As a class they are vegetable feeders, and these sharp teeth, arranged in rows upon the surface of the tongue, enable them to rasp away the surface of the succulent leaves upon which they feed. In the species from which the specimen was obtained which has furnished our photo-micrograph, it is estimated that the number of teeth exceeds twenty thousand.

Tracheæ of Insects. Plate XX. Fig. 2.

The tracheæ of Insects and of their larvæ are branching cylindrical tubes, which take their origin from the spiracles, which are apertures situated upon the lateral and upper portions of the abdomen, and at the posterior, lateral, and upper parts of the thorax. These branching tubes are distributed to all parts of the body, and serve to convey air to the fluids and tissues. They consist of two coats, between which lies a spiral fibre which is well seen in the photo-micrograph.

In conclusion, the Author ventures to hope that the elementary facts in biology and in histology

briefly outlined in this little volume may prove to be of sufficient interest to some of those into whose hands it may fall to induce them to seek fuller information in the "BOOK OF NATURE"; and that an inspection of the Plates may stimulate the curiosity of some of those who have not hitherto been interested in microscopical studies, and induce them to explore for themselves the broad domain of which I have here given them a brief glimpse.

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